47th Inner Ear Biology Workshop

Prague, Czech Republic
August 29 – September 1, 2010

Programme and Abstracts

Institute
of Experimental
Medicine AS CR, v.v.i.

Academy of Sciences
of the Czech Republic

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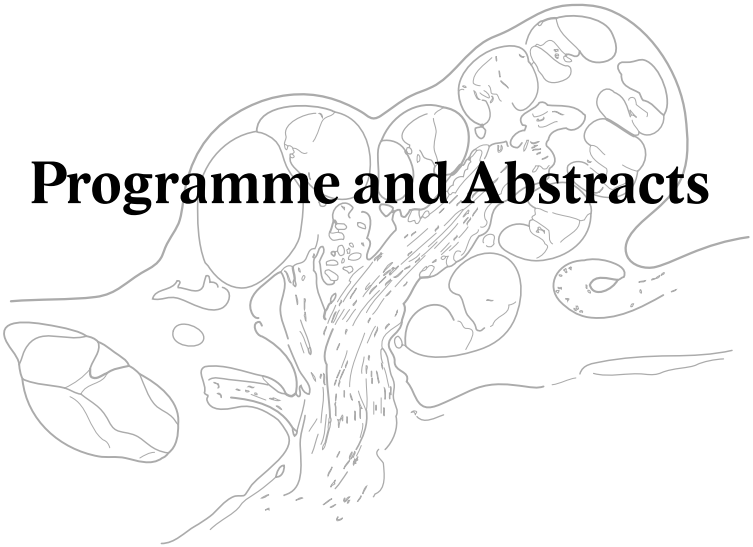

Programme and Abstracts

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of the Czech Republic

Dear Colleague,

The 47th Inner Ear Biology Workshop, a traditional European meeting, that attracts scientists interested in inner ear research from all over the world, is being held for the first time in Prague.

Active intellectual life in Prague can be traced back to 1348 when Charles University, the oldest university in Central Europe, was founded by the Czech King and Roman Emperor Charles IV. At the present time Charles University, with its 17 faculties and more than 30 thousand students, represents the leading Czech university in science. In addition to universities, science is actively pursued in the institutes of the Academy of Sciences and in several other research institutions. The Institute of Experimental Medicine of the Academy of Sciences, situated on the southern outskirts of Prague, is the leading institution in the Czech Republic for biomedical research. Among the fields of research traditionally pursued in the Institute is auditory research, with investigations of both the inner ear and the central auditory system. Research in sensory physiology and pathology has a strong tradition in the Czech Republic, starting with the many important contributions of the famous physiologist Jan Evangelista Purkyně in the 19th century. We also cannot forget the contribution of Ernst Mach to vestibular research during his stay in Prague and certainly not the groundbreaking findings in genetics of the monk Gregor Mendel, who lived in Brno, the second largest city of the Czech Republic. At the present time, the main goal of Czech science is to reach the same quality of research as performed in leading countries worldwide, thus enabling Czech researchers to contribute significantly to world science.

I hope you will have a chance, during or after the meeting, to appreciate the many beauties of Prague, particularly the ancient architectural monuments with styles ranging from romanesque, gothic, renaissance, baroque, classicism and art noveau up to modern styles. I cordially wish you an enjoyable stay in Prague and hope that you will return to your home enriched by many valuable experiences.

Josef Syka
Chairman of the Organising Committtee

Organising Committee

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Milan Jilek
Ladislav Ouda
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We would like to thank the following institutions and companies for supporting the Inner Ear Biology Workshop 2010:

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Conference Venue

The Workshop will be held in the conference rooms of the Hotel Diplomat, Evropská 15, Prague 6. The Hotel Diplomat is situated a short distance from several Prague universities, in a residential part of Prague, near the Prague Castle. Next to the hotel is a subway station.

Registration

The registration desk is located in the Hotel Diplomat, in the hall in front of the conference rooms, and will be open during the following hours:

Sunday, August 29	16:00 - 20:00
Monday, August 30	8:00 – 18:00
Tuesday, August 31	8:00 – 18:00
Wednesday, September 1	8:00 – 13:00

The registration fee includes: attendance of the scientific sessions, conference bag with abstract book and other materials, entrance to the Get Together party, lunches and coffee breaks during the conference.

Lunches and refreshments during the **coffee breaks** will be provided from Monday to Wednesday free of charge to registered participants in the Hotel Diplomat on the same level as the conference rooms.

A limited number of tickets for the **Beer Party Dinner** and the **Gala Dinner** will be availabe for purchase at the Registration Desk.

Posters No. 1 – 33 should remain posted throughout the entire day on Monday, while posters No. 34 – 68 should remain posted throughout the entire day on Tuesday. Presenting authors should be at their posters during all indicated poster presentation times. Poster board dimensions are 80 cm in width and 120 cm in height.

PROGRAMME OVERVIEW

Sunday 29th August

16:00 – 20:00	Registration
18:00 – 20:00	Get Together Party

Monday 30th August

08:00 – 18:00	Registration
09:00 – 09:15	Welcoming address
09:15 – 10:30	**Scientific session 1 – Genes and development I**
10:30 – 11:15	Coffee break and poster viewing
11:15 – 12:30	**Scientific session 2 – Genes and development II**
12:30 – 14:00	Lunch and poster viewing
14:00 – 15:30	**Scientific session 3 – Genes and development III**
15:30 – 16:15	Coffee break and poster viewing
16:15 – 17:15	**Scientific session 4 – Otoprotection and presbycusis**
20:00 – 23:00	Beer Party Dinner at The Strahov Monastery Restaurant *Location: The Strahov Monastery Restaurant* *Strahovské nádvoří 302, Prague 1*

Tuesday 31st August

08:00 – 18:00	Registration
09:00 – 10:30	**Scientific session 5 – Function of hair cells**
10:30 – 11:15	Coffee break and poster viewing
11:15 – 12:30	**Scientific session 6 – Audiovestibular research**
12:30 – 14:00	Lunch and poster viewing
14:00 – 15:30	**Scientific session 7 – Spiral ganglion neurons**
15:30 – 16:15	Coffee break and poster viewing
16:15 – 17:30	**Scientific session 8 – Cochlear pathologies I**
17:30 – 18:00	IEB Business meeting
20:00 – 23:00	Conference Gala Dinner at Kaiserštejnský Palác *Location: Kaiserstejnsky Palace, 2nd floor* *Malostranské namesti 23/37, Prague 1*

Wednesday 1st September

08:00 – 13:00	Registration
09:30 – 10:30	**Scientific session 9 – Cochlear pathologies II**
10:30 – 11:00	Coffee break
11:00 – 12:00	**Scientific session 10 – Varia**
12:00	End of the workshop
12:00 – 13:30	Lunch

PROGRAMME

Monday 30th August

9:00 Welcoming address

9:15 – 10:30 SCIENTIFIC SESSION 1. **Genes and development I**
Moderators: **J. Ashmore, F. Santos-Sacchi**

9:15 **O 01** **Combinatorial regulation of hair cell induction by transcription factors**
A. F. Ryan, M. Masuda, *La Jolla*

9:30 **O 02** **New insights into IGF-I actions in cochlear development and function**
L. Rodriguez de la Rosa, G. Camarero, R. Cediel, S. Murillo-Cuesta, I. Varela-Nieto, *Madrid*

9:45 **O 03** **Notch-Hes1 pathway contributes to the cochlear prosensory formation through the transcriptional down-regulation of p27Kip1**
J. Murata, T. Ohtsuka, A. Tokunaga, K. Kamiya, H. Inohara, K. Ikeda, H. Okano, R. Kageyama, *Osaka, Kyoto, Tokyo*

10:00 **O 04** **LIM-homeodomain Islet1 (ISL1) transcription factor in neurosensory development of the inner ear**
G. Pavlinkova, B. Fritsch, R. Bohuslavová, L. Kuthanova
Prague, Omaha

10:15 **O 05** **Cochlea supporting cell-specific ablation *in vivo* in mice**
M. M. M. Lagarde, B. Cox, L. Zhang, A. Forge, R. Taylor, J. Zuo
Memphis, London

10:30 – 11:15 Coffee break and poster viewing

11:15 – 12:30 SCIENTIFIC SESSION 2. **Genes and development II**
Moderators: **C. Hackney, I. Varela-Nieto**

11:15 **O 06** **Calcium binding proteins and the development of the inner ear in the mouse**
J. Syka, D. Buckiová, *Prague*

11:30 **O 07** **Abnormal structure and function of the intermediate-basal cell unit in stria vascularis of connexin30 null mice**
J. Kelly, A. Forge, D. Jagger, *London*

11:45 **O 08** **Disturbed postnatal development of the organ of Corti in dominant-negative Cx26 mutant mice**
K. Ikeda, A. Inoshita, A. Minekawa, K. Kamiya, T. Iizuka, *Tokyo*

12:00 **O 09** **Functional studies of the first knock-in mouse model for a connexin 30 mutation linked to nonsyndromic autosomal dominant deafness in humans (Cx30T5M)**
F. Mammano, M. Schütz, P. Scimemi, P. Majumder, R. D. De Siati, G. Crispino, L. Rodriguez, M. Bortolozzi, R. Santarelli, A. Seydel, S. Sonntag, N. Ingham, K. P. Steel, K. Willecke, *Padua, Bonn, Treviso, Cambridge*

12:15 **O 10** **Connexin 26 structure and function investigated in silico by Molecular Dynamics simulations**
F. Zonta, G. Zanotti, F. Mammano, *Padua*

12:30 – 14:00 Lunch and poster viewing

14:00 – 15:30 SCIENTIFIC SESSION 3. **Genes and development III**
Moderators: **A. Ryan, J. Schacht**

14:00 **O 11** **Development and pathology of the human otic capsule**
S. Soucek, L. Michaels, *London*

14:15 **O 12** **Expression pattern of Olig gene family in the developing inner ears**
N. Yamamoto, A. Yoshida, T. Nakagawa, J. Ito, *Kyoto*

14:30 **O 13** **Ush1c gene expression levels in the ear and eye suggest different roles for Ush1c in neurosensory organs in a new Ush1c knockout mouse**
C. Tian, X. Z. Liu, F. Han, H. Yu, C. Longo-Guess, B. Yang, F. Han, C. Lu, D. Yan, Q. Y. Zheng, *Cleveland*

14:45 **O 14** **Impaired and derailed sensory neurons in the inner ear of Slitrk6-deficient mice**
A. Zine, K.-I. Katayama, M. Ota, Y. Matsumoto, T. Inoue, J. Aruga, *Montpellier*

15:00 **O 15** **Tmprss3, a serine protease deficient in human DFNB8/10 deafness, is critical for the hair cell survival at the onset of hearing**
L. Fasquelle, H. Scott, M. Lenoir, J. Wang, G. Rebillard, S. Gaboyard, E. Neidhart, J.-L. Puel, M. Guipponi, B. Delprat, Geneva, Montpellier, *Adelaide*

15:15 **O 16** **The expression pattern and the function of Septins in the mouse cochlea**
A. Yoshida, N. Yamamoto, T. Nakagawa, J. Ito, *Kyoto*

15:30 – 16:15 Coffee break and poster viewing

16:15 – 17:15 SCIENTIFIC SESSION 4. **Otoprotection and presbycusis**
Moderators: **F. Nuttall, J. Pickles**

16:15 **O 17** **Contribution of IL6 and STAT3 to otoprotection**
A. J. Szczepek, Y. Yu, H. Olze, H. Haupt, B. Mazurek, *Berlin*

16:30 **O 18** **Trauma/inflammatory cascade-induced hair cell and hearing losses can be prevented by polymer- eluted dexamethasone**
T. R. Van de Water, E. Bas, C. T. Dinh, R. Abi-Hachem, S. Angeli, A. A. Eshraghi, *Miami*

16:45 **O 19** **Long-term antioxidant diet changes cochlear antioxidant capacity but not age-related hearing loss**
S.-H. Sha, M. A. Lauderdale, K. Halsey, K. Wearne, J. Schacht *Ann Arbor*

17:00 **O 20** **Adenosine kinase inhibition in the cochlea delays the onset of age-related hearing loss in C57BL/6J mice**
S. M. Vlajkovic, C. X. Guo, R. Telang, A. C. Y. Wong, V. Paramananthasivam, D. Boison, G. D. Housley, P. R. Thorne *Auckland, Sydney, Portland*

20:00 – 23:00 **Beer Party Dinner at the Strahov Monastery Restaurant**

Tuesday 31st August

9:00 – 10:30 SCIENTIFIC SESSION 5. **Function of hair cells**
Moderators: **F. Mammano, K. Ikeda**

9:00 **O 21** **Effect of chloride and membrane holding potential on OHCs: simultaneous measures of electromotility and nonlinear capacitance**
J. Santos-Sacchi, L. Song, *New Haven*

9:15 **O 22** **Mechanotransducer currents and resting potentials in cochlear outer hair cells**
M. Beurg, R. Fettiplace, S. L. Johnson, W. Marcotti, *Madison, Sheffield*

9:30 **O 23** **Analysis of transmembrane structure of prestin by single molecule force spectroscopy**
M. Murakoshi, T. Kawase, S. Kumano, H. Wada, *Sendai*

9:45 **O 24** **Identification of the electromotility motif in prestin, the motor protein of cochlear outer hair cells**
X. Tan, J. Pecka, J. Tang, O. Okoruwa, S. Lovas, K. Beisel, D. He, *Omaha*

10:00 **O 25** **Translocation of prestin having mutation in the GTSRH sequence by salicylate**
H. Wada, S. Kumano, M. Murakoshi, K. Iida, K. Ishihara, K. Tsumoto, K. Ikeda, T. Kobayashi, *Sendai*

10:15 **O 26** **Self-tuning and coupling between hair cells of the inner ear**
D. Bozovic, C. E. Strimbu, L. Fredrickson, *Los Angeles*

10:30 – 11:15 Coffee break and poster viewing

11:15 – 12:30 SCIENTIFIC SESSION 6. **Audiovestibular research**
Moderators: **S. Klis, T. Van de Water**

11:15 **O 27** **Somatostatin receptor-1 and -2 in the mammalian cochlea**
V. Radojevic, C. Setz, Y. Brand, A. Lystio, J. Kapfhammer, D. Bodmer, *Basel*

11:30 **O 28** **A new approach for semi-automatic segmentation of the cochlea from CT images**
A. A. Poznyakovskiy, Y. M. Yarin, Y. Kalaidzidis, B. Fischer, N. Lazulashvili, T. Zahnert, *Dresden*

11:45 **O 29** **Glutamate agonist causes irreversible degeneration of inner hair cells**
N. Hakuba, J. Hyodo, M. Okada, Y. Omotehara, K. Gyo, *Matsuyama*

12:00 **O 30** **A re-examination of the striated organelle in vestibular endorgans**
F. Vranceanu, A. Lysakowski, *Chicago*

12:15 **O 31** **Morphological change of the cupula and its effect on the semicircular canal activity**
M. Suzuki, U. Konomi, K. Otsuka, Y. Iimura, T. Inagaki, T. Kondo, Y. Ogawa, *Tokyo*

12:30 – 14:00 Lunch and poster viewing

14:00 – 15:30 SCIENTIFIC SESSION 7. **Spiral ganglion neurons**
Moderators: **R. Fettiplace, M. Knipper**

14:00 **O 32** **Myelination and protein expression associated with human spiral ganglion neuron preservation**
W. Liu, M. Boström, A. Kinnefors, F. Linthicum, H. Rask-Andersen, *Uppsala, Los Angeles*

14:15 **O 33** **Voltage-dependent ion conductances in long-term cultures of rat spiral ganglion neurons**
D. McAlpine, P. Mistrík, D. Jagger, *London*

14:30 **O 34** **Intrinsic determinants of enhanced mid-frequency sensitivity in murine spiral ganglion neurons**
Q. Liu, E. Lee, R. L. Davis, *New Jersey*

14:45 **O 35** **Effects of electrical stimulation on the acoustically evoked compound action potential**
H. C. Stronks, H. Versnel, V. F. Prijs, S. F. L. Klis, *Utrecht*

15:00 **O 36** **The transcriptional response of spiral ganglion neurons to deafferentation is partially ameliorated by acute electrical stimulation**
J. C. Kopelovich, E. Bailey, J. R. Manak, S. Butcher, S. H. Green, *Iowa City*

15:15 **O 37** **The expansive relation between instantaneous rate of period histograms and instantaneous pressure of pure tones is retained at high stimulus levels**
J. W. Horst, J. McGee, E. J. Walsh, *Groningen, Omaha*

15:30 – 16:15 Coffee break and poster viewing

16:15 – 17:30 SCIENTIFIC SESSION 8. **Cochlear pathologies I**
Moderators: **B. Lonsbury-Martin, J. Popelář**

16:15 **O 38** **Cochlear repair and hair cells regeneration after human pluripotent stem cell transplantation to nod-scid mice made deaf with kanamycin and intense noise**
R. P. Revoltella, V. Franceschini, R. Saccardi, S. Urbani, B. Mazzanti, A. Martini, *Pisa, Bologna, Florence, Padua*

16:30 **O 39** **An *in vitro* model of synapse degeneration and regeneration in the cochlea**
Q. Wang, S. H. Green, *Iowa City*

16:45 **O 40** **A role for Hensen cells in the resolution of inflammatory responses in the cochlea**
A. Maricle, G. Kalinec, D. Guerrero, R. Gellibolian, P. Webster, F. Kalinec, *Los Angeles*

17:00 **O 41** **Matrix metalloproteinases -2 and -9 in the cochlea: expression and activity after aminoglycoside exposition**
C. Setz, Y. Brand, V. Radojevic, C. Hanusek, P. J. Mullen, S. Levano, A. Listyo, D. Bodmer, *Basel*

17:15 **O 42** **Ototoxicity and bacteriostatic activity of Burow's solution**
T. Morizono, T. Yamano, M. Sugamura, H. Higuchi, T. Ueno, T. Nakagawa, *Fukuoka*

17:30 – 18:00 **Business meeting**

20:00 – 23:00 **Gala Dinner at the Kaiserštejnský Palác**

Wednesday 1st September

9:30 – 10:30 SCIENTIFIC SESSION 9. **Cochlear pathologies II**
Moderators: **D. Mc Alpine, A. Meyer zum Gottesberge**

9:30 **O 43** **Evaluation of hyperbranched Poly-L-Lysine uptake and toxicity on inner ear derived cell line as a function of concentration and architecture**
E. Corbacella, Z. Kadlecova, L. Astolfi, H. A. Klok, A. Martini *Ferrara, Lausanne, Padua*

9:45 **O 44** **Manufacturing and in vivo inner ear visualization of MRI traceable multifunctional liposome nanoparticles tagged by gadolinium**
J. Zou, R. Sood, S. Ranjan, D. Poe, U. A. Ramadan, P. Kinnunen, I. Pyykkö, *Tampere, Helsinki*

10:00 **O 45** **The passage of nanoparticles from the middle ear to the cochlea in the rat**
D. Buckiová, J. Popelář, T. Chumak, J. Syka, *Prague*

10:15 **O 46** **Cause of sudden hearing loss (Where's the energy?)**
G. Offutt, *Green Lane*

10:30 – 11:00 Coffee break

11:00 – 12:00 SCIENTIFIC SESSION 10. **Varia**
Moderators: **G. Martin, J. Syka**

11:00 **O 47** **A CCTTT-repeat at the promoter of NOS2A gene confers protection to Ménière`s disease in two cohorts of European population**
J. A. Lopez-Escamez, A. Moreno, I. Gazquez, I. Aran, A. Soto-Varela, S. Santos, H. Perez-Garrigues, M. A Lopez-Nevot, *Almería*

11:15 **O 48** **Two Portuguese cochlear implanted dizygotic twins: a case report**
J. Chora, T. Matos, J. Martins, M. Alves, R. Santos, L. Silva, C. Ribeiro, G. Fialho, H. Caria, *Lisbon, Coimbra, Setúbal*

11:30 **O 49** **The GJB2 gene and hearing loss: beyond the coding region**
T. D. Matos, H. Simões-Teixeira, H. Caria, A. R. Rodrigues, H. Rosa, A. O´Neill, M. E.Andrea, D. P. Kelsell, G. Fialho
Lisbon, Setúbal, Almada, London

11:45 **O 50** **Cerebrocortical plasticity after acute and chronic unilateral lesion of the central vestibular system in rats: a micro-PET study**
E. Lange, H. G. Buchholz, N. Bausbacher, A. Kronfeld, U. Stier, C. Best, M. Dieterich, M. Schreckenberger, S. Reuss
Mainz, München

12:00 **End of the workshop**

12:00 – 13:30 Lunch

POSTER PRESENTATION

Monday 30th August

P 01 **Microarray analysis of differential gene expression along the tonotopic axis of mouse cochlea**
E. J. Son, S. Kim, L. Wu, H. Kim, J. Bok, *Seoul*

P 02 **TMC1-Associated deafness in the Portuguese population**
H. Simões-Teixeira, F. Moreno, G. Fialho, I. Del Castillo, H. Caria
Lisbon

P 03 **Screening of mitochondrial DNA mutations involved in hearing impairment**
V. Guaran, A. Castiglione, E. Tosi, M. Galasso, S. Volinia, A. Martini
Ferrara, Padua

P 04 **Connexin 26 and connexin 30 immunostaining in wild type and Cx30T5M knock-in mice**
G. Crispino, F. Mammano, *Padua*

P 05 **Generation of a tamoxifen inducible hair cell specific TR(beta)1 knock-out mouse model**
J. Dettling, C. Franz, U. Zimmermann, L. Rüttiger, J. Zuo, R. Feil, F. Flamant, M. Knipper, *Tübingen, Memphis, Lyon*

P 06 **Intracochlear injection of adeno-associate virus vector to a mouse model created by a conditional knockout of GJB2 gene**
T. Iizuka, H. Mochizuki, H. Okada, K. Kamiya, O. Minowa, T. Noda, K. Ikeda, *Tokyo*

P 07 **Cochlear gap-junction plaque is disrupted by dominant-negative connexin26 mutation**
K. Kamiya, K. Ikeda, *Tokyo*

P 08 **Role of neural crest and Pax3 in mammalian inner ear development**
D. J. Lee, K.-A. Kong, J. Bok, *Seoul*

P 09 **Role of RAF kinases in inner ear development**
M. Magariños, Maria Rodríguez Aburto, Rocio de Iriarte Rodríguez, U. Rapp, I. Varela-Nieto, *Madrid, Munich*

P 10 **Differential requirement of Pou3f4 in the development of cochlear lateral wall: A study on a mouse model for DFN3**
L. Wu, M. H. Song, S.-Y. Choi, S.-K. Oh, H. K. Lee, D. B. Shim, J. Y. Choi, U.-K. Kim, J. Bok, *Seoul, Gangweon, Taegu*

P 11 **Expression analysis of prestin and selected transcription factors in newborn rats**
J. Gross, M. Angerstein, J. Fuchs, K. Stute, B. Mazurek, *Berlin*

P 12 **Immunological and genetical influence of systemic helper T cell on age-related cochlear functions**
H. Iwai, M. Inaba, S. Baba, M. Sakaguchi, S. Ikehara, K. Tomoda, *Osaka*

P 13 **Degeneration in marginal cells of the ageing mouse stria vascularis**
J. O. Pickles, *Brisbane*

P 14 **Activation of NF-κB-iNOS pathway in the lateral wall of aged cochlea**
K.-I. Watanabe, S. Inai, K. Ohkubo, *Tokyo*

P 15 **Identification of Caprin-1 as a novel target of Pou4f3 and its role in cochlear hair cells in response to ototoxic damage**
E. R. Towers, J. J. Kelly, R. Sud, J. E. Gale, S. J. Dawson, *London*

P 16 **Evaluation of the potential meningitis risk of dexamethasone-eluting cochlear implants: A new animal model**
K. Niedermeier, T. Stark, S. Braun, R. Straubinger, J. Kiefer, A. Lohner, U. Koedel, S. Hammerschmidt, C. Fauser, *Munich*

P 17 **Ototoxicity and bacteriostatic activity of methylrosaniline chloride**
H. Higuchi, T. Yamano, M. Sugamura, T. Ueno, T. Nakagawa, T. Morizono, *Fukuoka*

P 18 **Cross-defense of auditory hair cells against ototoxicity of gentamicin by non-toxic levels of amikacin**
J. A. A. de Oliveira, D. A. E. Pires, M. Â. Hyppolito, M. Rossato *Ribeirão Preto*

P 19 **Synaptic activity in turtle calyx endings**
S. Chatlani, J. M. Goldberg, *Chicago*

P 20 **Electrophysiological and anatomical characterization of developing human cristae**
R. Lim, A. J. Camp, M. A. Walsh, R. J. Callister, A. M. Brichta, *Callaghan*

P 21 **Damage-induced ERK1/2 activation in mouse utricular organ explants and MDCK monolayers**
G. J. Ball, J. E. Gale, *London*

P 22 **Potassium and calcium concentration in cochlear endolymph after vestibular labyrinth injury**
R. Ikeda, K. Nakaya, M. Yamazaki, T. Oshima, T. Kawase, T. Kobayashi *Sendai*

P 23 **Injection of virus vector targeting vestibule in mice**
H. Okada, T. Iizuka, K. Kamiya, M. Kasai, H. Kasagi, A. Inoshita, K. Ikeda, *Tokyo*

P 24 **Molecular identity and cAMP modulation of the hyperpolarization-activated current in afferent vestibular neurons**
A. Almanza, F. Mercado, E. Luis, R. Vega, E. Soto, *Puebla*

P 25 **Neurofibrillary pathology in the vestibular nuclei of the pR5 mouse**
H. Schröder, C. Köhler, K. Pilz, T. Bauer, A. Selzer, E. Lange, J. Götz, S. Reuss, *Cologne, Mainz, Sydney*

P 26 **Type 1 allergy-induced endolymphatic hydrops and the suppresive effect of anti-histamine drugs**
T. Takeda, S. Takeda, A. Kakigi, R. Nishioka, *Nishinomiya, Tokyo, Kochi*

P 27 **Expression of the proinflammatory cytokines in cochlear explant cultures: influence of normoxia and hypoxia**
H. Haupt, A. J. Szczepek, M. Khan, H. Olze, B. Mazurek, *Berlin*

P 28 **Detection of drugs in cochlear fluids following round window membrane application in the rat**
H. Xiong, L. Rüttiger, A. Zuccotti, W. Singer, H.-S. Geisler, M. Knipper, *Tübingen*

P 29 **Intracochlear distribution of intratympanically injected quantum dot-dexamethasone nanocomplex and analysis of the hearing recovery according to the frequency in sudden sensorineural hearing loss**
I.-W. Lee, J. Lee, S.-K. Kong, E.-K. Goh, *Busan*

P 30 **Increase of the neuroprotective effect of rolipram on spiral ganglion cells via nanoparticles carriage**
H. Meyer, T. Stöver, F. Fouchet, G. Bastiat, P. Saulnier, W. Bäumer, T. Lenarz, V. Scheper, *Hannover, Angers, Frankfurt*

P 31 **Nanoparticle-silicone composites for reduction of fibrous tissue growth**
A. Burghard, A. Hahn, S. Barcikowski, H. Rohm, K. Sternberg, T. Lenarz, T. Stöver, G. Paasche, *Hannover, Rostock*

P 32 **Intracochlear distribution of hyperbranched poly-L-lysine after intratympanic application in mice**
T. Chumak, Z. Kadlecova, H.-A. Klok, J. Popelář, J. Syka
Prague, Lausanne

P 33 **The effect of topical sodium thiosulfate in experimentally induced myringosclerosis**
Y.-H. Park, *Daejeon*

Tuesday 31st August

P 34 **Analysis of the head related impulse response by means of an auditory model**
F. Rund, V. Vencovský, *Prague*

P 35 **Calculation of the traveling wave velocity - effects on sound coding**
P. Marsalek, F. Julicher, *Dresden, Kladno*

P 36 **Biological applications to the acoustic-wave hearing's model**
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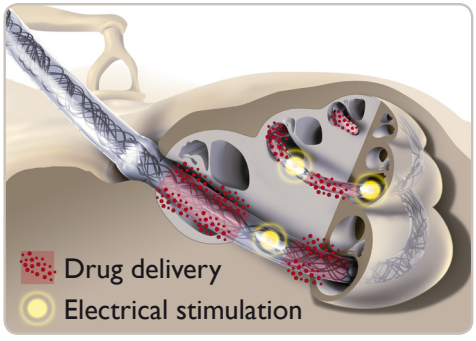

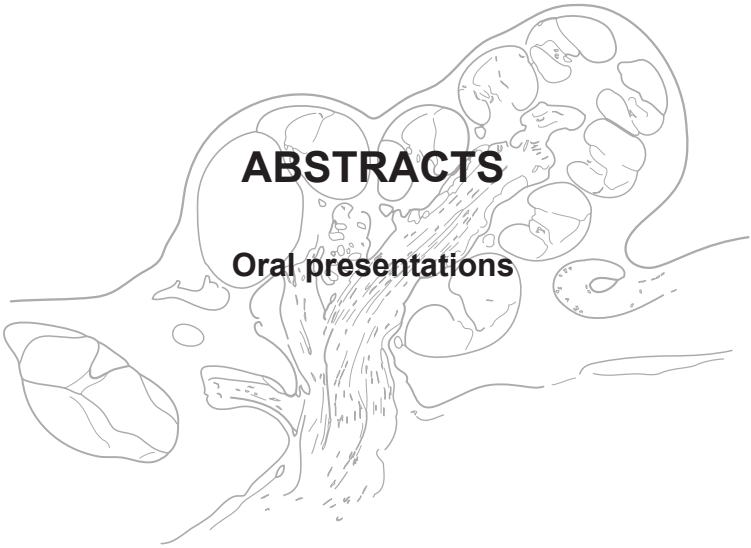

ABSTRACTS

Oral presentations

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Combinatorial regulation of hair cell induction by transcription factors

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Forced expression of the basic helix-loop-helix transcription factor Atoh1 has been shown to induce the transformation of supporting cells in the organ of Corti into hair cells (HCs). Evaluating the regulation of the pou4f3 gene, a likely target of Atoh1 in HCs, we identified a cluster of binding sites for Atoh1 and several other transcription factors (TFs), a feature that was highly conserved across mammalian species. To test the hypothesis that these TFs act in combination to regulate the pou4f3 gene we transfected, by electroporation, neonatal mouse organ of Corti with plasmids encoding the transcription factors Atoh1, E47, GATA3 and Sp1 in various combinations. We used a transgenic mouse in which 5' DNA from the pou4f3 ATG drives expression of GFP to assess the regulation of this gene, and labeled the cultures with anti-myosin VIIA to identify HCs. Transfection of Atoh1 alone induced expression of GFP in supporting cells, the majority of which were located in the greater epithelial ridge (GER). Most of these cells also expressed myosin VIIA, indicating conversion to a HC phenotype. E47 alone also induced GFP expression in many GER cells, but not that of myosin VIIA. Neither GATA3 or SP1 alone induced expression of either GFP or myosin VIIA. Co-transfection of Atoh1 with either E47 or GATA3 produced a 2-3 fold increase the number of cells expressing GFP, and a corresponding increase in cells expressing myosin VIIA, when compared to Atoh1 alone. Co-transfection of Atoh1 with Sp1 was comparable to Atoh1 alone. The results suggest that Atoh1 acts in concert with other transcription factors to control the expression of the pou4f3 gene, and more generally to induce a HC phenotype. These factors could be used to enhance HC regeneration.

New insights into IGF-I actions in cochlear development and function

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Insulin-like growth factor I (IGF-I) mutations are associated with deafness in mice and men but little is known about the molecular basis of this condition. IGF-I actions are mediated by intracellular signaling networks primarily activated by the phosphorylation of insulin receptor substrates (IRS2) and down-regulated by the tyrosine phosphatase PTP1B. In the embryonic and neonatal mice, Igf1 is expressed in a pattern that is complementary to that of its high affinity receptor. IGF-I deficit causes the down regulation of ERK and AKT pathways and the activation of the p38 stress kinase. RNA arrays experiments evidenced that transcription factors FoxM1 and MEF2A and D are novel cochlear targets for IGF-I. In the adult mouse, levels of IGF-I decrease during ageing, a trait that associates with age-related hearing loss. Auditory brainstem responses (ABR) thresholds in Irs2-/- null mice were higher than those of wild types whereas Ptp1b-/- null mice did not show any evident functional nor morphological difference with the control mice. Young Irs2-/- mice showed a decreased width of the stria vascularis, an abnormal dilatation of strial vasculature and a reduction in the ganglion size compared with the wild type mouse. Similarly, the Igf1-/- null mice presents an early neuronal degeneration and a prematurely aged stria vascularis also reminiscent of the diabetic strial phenotype. In conclusion, IGF-I signaling is required for the correct development and maintenance of hearing, supporting the idea that IGF-I-based therapies could contribute to prevent or ameliorate age-related hearing loss. Sanchez-Calderon et al. PLoS One. 2010 ; Riquelme et al. Frontiers Neuroanatomy, 2010.

Notch-Hes1 pathway contributes to the cochlear prosensory formation through the transcriptional down-regulation of p27Kip1

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The Notch signaling pathway has a crucial role in the differentiation of hair cells and supporting cells by mediating “lateral inhibition” via the ligands Delta–like1 (Dll1) and Jagged2 (Jag2) and the effectors Hes1 and Hes5 during mammalian inner ear development. Recently, another Notch ligand Jagged1 (Jag1)-dependent Notch activation has been revealed to be important for the determination of the prosensory region in the earlier stage before the cell differentiation. However, little is known about the effectors of the Notch pathway in this context. P27Kip1, a cyclin-dependent kinase inhibitor, is also known to demarcate the prosensory region in the cochlear primordium, which consists of the sensory progenitors that have completed their terminal mitoses. Hes1 reportedly promotes the precursor cell proliferation through the transcriptional down-regulation of p27Kip1 in the thymus, liver, and brain. In this study, we noticed Hes1 as a mediator between Notch signaling pathway and the regulation of the proliferation of sensory precursor cells by p27Kip1 in the developing cochlea. We showed that Hes1, not Hes5 was weakly expressed at the time of onset of p27Kip1. The expression pattern of Hes1 prior to the cell differentiation was similar to that of activated Notch1. P27Kip1 was up- regulated and BrdU-positive S-phase cells were reduced in the developing cochlear epithelium of Hes1-null mice. These results suggest that Notch-Hes1 pathway may contribute to the adequate proliferation of sensory precursor cells via the transcriptional down-regulation of p27Kip1 expression, and play a pivotal role in the correct prosensory determination. This work was supported by a grant to J.M. from the Japanese Ministry of Education, Culture, Sports, Science, and Technology.

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LIM-homeodomain Islet1 (ISL1) transcription factor in neurosensory development of the inner ear

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Approximately 71 million Europeans have a hearing impairment. Approximately one-half of these cases are thought to be of hereditary origin. Hearing loss can be conductive or neurosensory or both (mixed loss). Conductive hearing loss occurs when sound is not transmitted efficiently through the outer and middle ear. It can often be medically or surgically corrected. Neurosensory hearing loss results from the death of hair cells, supporting cells, neurons or the loss of neuronal contacts in the inner ear (cochlea). Like neurons in general, these cells have little ability to regenerate; therefore, their damage results in permanent hearing loss. Several transcription factors have been shown to be essential for the generation of auditory sensory hair cells or spiral ganglion neurons. However, cellular context-dependent mechanisms that confer inner ear-specific neuronal or sensory identities are elusive. Using mouse model and in situ hybridization, we show that Isl1 is expressed early during ear development in the otocyst and delaminating sensory neuron precursors at E9.5. We hypothesize that Islet1, which is expressed in the precursors that give rise to neurons and sensory epithelia, plays a role in the cell fate decision of neurosensory cells in the inner ear. We established the earliest expression pattern of Isl1 during ear development, utilizing Isl1-Cre/+ expression in the R26R-lacZ line. R26R-lacZ mice are reporter strains and are used to test the tissue/cellular expression pattern of the Cre transgene in any transgenic strain carrying Cre under the regulation of a specific promoter. Cre expression results in the removal of a loxP-flanked DNA segment that prevents the expression of a lacZ gene. Successful Cre excision is indicated by β-galactosidase expression in Cre+ tissues. Our lineage studies have shown that this Cre line recapitulates the expression of the endogenous Isl1 gene during ear development, as visualized by in situ hybridization. Our expression data show that Isl1 is expressed early in the otocyst, which supports our hypothesis that Isl1 plays a role in the neurosensory development of the inner ear. To test whether ISL1 plays a key role in regulating the selection and population size of neurosensory precursors, we misexpressed Isl1 under the control of Pax2 promoter. Pax2 is expressed in all cells of the otic placode, whereas Isl1 is expressed in a few cells within the otic epithelium at E9.5. Therefore, Pax2 promoter effectively expands Isl1+ domain in transgenic mice. Our future experiments, using Pax2-Isl1 transgenic mouse mutants, will address ISL1 functional role in the neurosensory development of the inner ear.

Cochlea supporting cell-specific ablation *in vivo* in mice

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Hair cells in the organ of Corti are inserted in a very well structured environment formed by supporting cells and acellular matrixes. Cochlea supporting cells (SCs) are responsible for sealing the epithelial surface when hair cells are irreversibly damaged in mammals and their maintenance determines the survival of the auditory neurons. SCs also play a key role in cochlear ionic balance; they express the gap-junction protein connexin 26, whose mutations account for about 50% of congenital deafness in humans. Up-to-date it is not known how primary loss of cochlear SCs affects cochlear development and function. In this study we aim to specifically ablate two types of cochlear supporting cells that have very specialized architecture, pillar and Deiters' cells, to determine the effects of this ablation on the morphological development and function of the organ of Corti. We used the mouse line Prox1CreERT2, in which Cre can be selectively induced in pillar and Deiters cells postnatally. These CreER mice were bred with floxed mice in which Diphteria Toxin fragment A (DTA) is expressed after Cre-mediated deletion of a stop-codon (Rosa26-eGFP-DTA). We injected Prox1CreER/+;DTAloxp/+ and control mice with tamoxifen at P0 and P1, collected cochleae at P2, P4, P8, P15, P25 and 8 weeks, and measured auditory brainstem responses (ABR) at P25. SC loss in Prox1CreER/+;DTAloxp/+ mice is evident at P2 and progresses up to P25 while hair cell loss (outer hair cells) is evident from P15. At P25 and up to 8 weeks almost all outer hair cells are lost while inner hair cells remain preserved. Besides the significant loss of SCs and hair cells the epithelium seems to preserve its ability to seal at the surface. ABRs can be recorded in Prox1CreER/+;DTAloxp/+ mice at P25 although their thresholds are 40dB higher than those of control littermates. Neuronal innervation to the outer hair cell area is disrupted in Prox1CreER/+;DTAloxp/+ mice from P8. These results show that the integrity of pillar and Deiters cells is crucial to hair cell survival and neural preservation in the organ of Corti.

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Calcium binding proteins and the development of the inner ear in the mouse

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In a recent study (Buckiová and Syka, 2009) we characterized calbindin (CB) and S100 expression in the developing inner ear in mice starting from the early embryonic days up to postnatal day 10. The aim of this report is to complete our overview of the expression of calcium binding proteins (CBP) in the inner ear of mice by adding information about the developmental expression of two other members of the CBP family – parvalbumin α (PV) and calretinin (CR) – from embryonic day 11 (E11) to postnatal day 21 (P21). Using paraffin sections and immunohistochemistry, their occurrence was investigated in the cochlea and in the vestibular system. Double immunofluorescence was utilized to determine whether their appearance is independent of one another or if there is some colocalization in their expression. For visualization, a Zeiss 510 confocal microscope was used.

The first of the studied CBP, calbindin, appeared in the otocyst and vestibulocochlear ganglion at E11. It was expressed throughout the whole embryonic period of development, reaching a peak early postnatally, and remained present in adult animals. S 100 immunoreactivity started in the vestibular duct at E14. Distinct immunostaining was observed in the cochlea from E17. Postnatally, S100 activity was restricted to the spiral ganglion. The first signs of the occurrence of CR were detected at E17 in both the vestibular system and the cochlea, where CR was present in the inner hair cells. CR immunoreactivity was also observed in the spiral ganglion and persisted to adulthood.

PV immunoreactivity appeared later than in the case of the other CBF – at E19. PV was expressed in both the inner and outer hair cells and in the spiral ganglion. PV remained strongly expressed into adulthood.

The colocalization of CB and CR was found during development in the inner hair cells starting from E17. Subsequently, (E18-19) CR immunoreactivity was present throughout the whole cell, while the occurrence of CB was concentrated at the base and apex of the inner hair cells. Spiral ganglion neurons were stained by either CR or by CB exclusively. However, after day 5 CB expression predominated in most spiral ganglion cells. The colocalization of PV with CR was found to be very limited.

D. Buckiová and J. Syka, J. Comp. Neurol. 513: 469-482, 2009.

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Abnormal structure and function of the intermediate-basal cell unit in stria vascularis of connexin30 null mice

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Connexin26 (Cx26) and Cx30 are the predominant connexins in the cochlea. Both are essential for hearing. Mutations in either connexin gene cause deafness, though the cochlear function of these proteins remains elusive. This question is complicated by the distinct pathologies caused by knockout or mutations of either connexin. Conditional Cx26-/- mice and a knock-in of a human dominant mutation (R75W) suffer severe deafness and have delayed cochlear maturation, but the endocochlear potential (EP) develops normally. However, Cx30-/- mice are reported to develop normally but never generate an EP. Given the expected non-selective properties of gap junctions it is surprising that one connexin cannot compensate for the loss of the other, and this points to distinct functions for Cx26 and Cx30 in both cochlear development and production of EP. We have set out, therefore, to determine the contribution of Cx30 to the EP generating machinery. Contrary to previous reports (Teubner et al, Hum Mol Gen 2003; Cohen-Salmon et al, PNAS 2007), we have observed a relative thinning of stria vascularis (SV) in Cx30-/- mice compared to wild-type (WT) littermates. The organization of SV appeared less distinct in Cx30-/- mice, with a reduced intra-strial space and decreased numbers of inter-digitations of intermediate cells with marginal cells. The expression of proteins involved in EP production was also analyzed. Immuno-fluorescence for Kir4.1, a potassium channel expressed in intermediate cells, was noticeably lower in Cx30-/- mice. Real-time quantitative PCR of SV revealed that Kir4.1 mRNA in Cx30-/- was around 75% of that in WT mice. In marginal cells, immuno-fluorescence for NKCC1 (an ion co-transporter) and Na-K-ATPase appeared comparable between Cx30-/- and WT mice. In basal cells, immuno-fluorescence for Claudin-11 (a tight junction protein) and GLUT-1 (glucose transporter) appeared normal in Cx30-/- mice, but there was a marked reduction of the F-actin content in these cells. In the spiral ligament and SV immuno-fluorescence for Cx26 appeared similar in localisation and intensity between Cx30-/- and WT mice. It has been suggested that in Cx30-/- mice SV capillaries display an increased protein permeability (Cohen-Salmon et al, PNAS 2007). We assessed this possibility by intra-peritoneal injection of bumetanide (a loop diuretic that blocks NKCC co-transporters in marginal cells) into both WT and Cx30-/- mice. This procedure normally causes oedema and consequent swelling within the intra-strial space. Bumetanide caused intra-strial swellings in WT and Cx30-/- mice, suggesting that the capillary integrity in both animals was normal. Also, FITC-conjugated bovine serum albumin injected into tail-veins (which identifies capillary leakage in wound healing) revealed no leakage within SV of WT or Cx30-/- mice. These experiments suggest that the endothelial barrier is intact in Cx30-/- mice. Our results suggest that the lack of an EP in Cx30-/- mice may result from abnormal development of the functional unit composed of gap junction-coupled intermediate and basal cells.

Disturbed postnatal development of the organ of Corti in dominant-negative Cx26 mutant mice

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Hereditary hearing loss is one of the most prevalent inherited human birth defects, affecting 1 in 2,000. Although a strikingly high proportion (50%) of congenital bilateral nonsyndromic sensorineural deafness cases have been linked to mutations in the GJB2 coding for the connexin26, the underlying mechanisms of GJB2-based deafness has not fully clarified. We previously reported the generation of a mouse model carrying human connexin26 with R75W mutation (Kudo et al, Hum Mol Genet 2003). In the present study, we attempted to evaluate postnatal development of the organ of Corti in the R75W+ mice. R75W+ mice have never shown ABR waveforms throughout postnatal development, indicating the disturbance of auditory organ development. Histological observations at P5-14 were characterized by i) no formation of tunnel of Corti, Nuel's space, or spaces surrounding the outer hair cells or, ii) significantly small numbers of microtubules in inner pillar cells, iii) shortening of height of the organ of Corti, and vi) increase of the cross-sectional area of the cells of the organ of Corti. The total number of the greater epithelial ridge (GER) cell and apoptotic cells had decreased since P10 in wild mice. On the other hand, the total number of GER and apoptosis in GER cells were presented at P10 and P12 in R75W+ mice. The present of GER may be due to diminish apoptosis and/or promotion of cell division during embryogenesis and postnatal periods, owing to the Cx26 with R75W+ mutation. We demonstrated the delayed apoptosis in GER cells and enlarged area of GER in the R75W+ mouse. Thus, morphological observations confirmed that a dominant-negative Gjb2 mutation showed incomplete and abnormal development of the cochlear supporting cells (Inoshita et al., Neuroscience 2008). No detectable distortion product otoacoustic emissions were observed at any frequencies in R75W transgenic mice throughout development. However, the OHC of the R75W+ mice were compressed and squeezed by the surrounding supporting cells. On the other hand, the OHC developed normally. Structural features of the lateral wall, such as the membrane-bound subsurface cisterna beneath the plasma membrane, were intact. Prestin, the voltage-dependent motor protein, was observed by immunohistochemistry in the OHC basolateral membranes of both transgenic and non-transgenic mice. No significant differences in electromotility of isolated OHCs during development were observed between transgenic and control mice (Minekawa et al., Neuroscience 2010). The present study indicates that normal development of the supporting cells is indispensable for proper cellular function of the OHC. The present study suggests that Gjb2 is indispensable in the postnatal development of the organ of Corti and normal hearing.

Functional studies of the first knock-in mouse model for a connexin 30 mutation linked to nonsyndromic autosomal dominant deafness in humans (Cx30T5M)

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Single strand conformation polymorphism (SSCP) mutational analysis in 198 deaf patients, including 38 families linked to chromosome 13q12, revealed a threonine-to-methionine change at position 5 (T5M) of the GJB6 gene, encoding connexin 30 (Cx30), in an Italian family affected by bilateral middle/high-frequency hearing loss [1]. The Cx30T5M mutation was inserted in the mouse genome by homologous recombination in mouse embryonic stem (ES) cells. Thresholds determined by auditory brainstem recordings (ABR) were moderately (about 15 dB) albeit significantly higher in $Cx30^{T5M/T5M}$ mice relative to $Cx30^{+/+}$ mice ($p < 0.005$), whereas we found no differences between $Cx30^{+/T5M}$ and $Cx30^{+/+}$ mice. This result is in contrast to the report that heterozygous human carriers of the Cx30T5M mutation are deaf [1]. On P30, immunolabelling with antibodies specific for Cx30 or Cx26 produced patterns that were similar, but not identical, for Cx26 and Cx30. However, no discernible differences between $Cx30^{+/+}$, $Cx30^{+/T5M}$ and $Cx30^{T5M/T5M}$ mice were noticed. By contrast, our results show that decreased dye transfer and ATP release [2] in immature $Cx30^{T5M}$ cochleae is associated with mild increase in hearing threshold. Assuming that the $Cx30^{T5M}$ mutation does not affect electrical coupling in the cochlea, based on ref. [3], our findings support the notion that impaired biochemical coupling can cause defective hearing in mice and man. A key role might be played, at the developmental level, by spontanous activity in inner sulcus cells [4, 5], which is significantly decreased in cochlear cultures from P5 $Cx30^{T5M/T5M}$ mice compared to $Cx30^{+/+}$ controls.

1. Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, Rabionet R, Arbones M, Monica MD, Estivill X, Zelante L, Lang F, Gasparini P. (1999), Nat Genet, 23, 16-8.
2. Anselmi F, Hernandez VH, Crispino G, Seydel A, Ortolano S, Roper SD, Kessaris N, Richardson W, Rickheit G, Filippov MA, Monyer H, Mammano F. (2008), Proc Natl Acad Sci USA, 105, 18770-5.
3. Zhang Y, Tang W, Ahmad S, Sipp JA, Chen P, Lin X. (2005), Proc Natl Acad Sci USA, 102, 15201-6.
4. Tritsch NX, Yi E, Gale JE, Glowatzki E, Bergles DE. (2007), Nature, 450, 50-5.
5. Tritsch NX, Bergles DE. (2010), J Neurosci, 30, 1539-50.

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Connexin 26 structure and function investigated in silico by Molecular Dynamics simulations

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About half of all cases of human deafness in countries surrounding the Mediterranean have been linked to mutations in the GIB2 gene, which encodes the gap junction protein connexin 26 (Cx26) (1). We chose a Molecular Dynamics approach aiming to construct a computational environment for the interpretation of experimental results and the prediction of effects not yet observed. Starting from the recently published crystal structure of the Cx26 gap junction channel (2), we built a fully atomistic model of the Cx26 connexon including plasma membrane phospholipids, water and ions (the whole system contains 206188 atoms) and followed its equilibrium dynamics for about 13 ns. This in silico approach enlivens the "frozen" crystallographic structure of Cx26 and permits us to study the protein in a realistically simulated environment. The analysis of temporal trajectories of each molecule provides critical insight into the interactions that shape the tertiary and quaternary structure of the protein complex. In addition, we can simulate equilibrium thermal fluctuation of the protein and study the dynamics of ions and secondary messenger transit through the pore, which provides invaluable mechanistic insight into channel function and dysfunction, including permeation and gating mechanisms.

1. Petit, C., Levilliers, J., and Hardelin, J. P. (2001) Annu Rev Genet 35, 589-646
2. Maeda, S., Nakagawa, S., Suga, M., Yamashita, E., Oshima, A., Fujiyoshi, Y., and Tsukihara, T. (2009) Nature 458, 597-602

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Development and pathology of the human otic capsule

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We observed marked changes in the portion of the otic capsule immediately surrounding the endolymphatic duct, the bony intravestibular extension of the vestibular aqueduct, in cases of Meniere's disease. In order adequately to interpret these changes we studied the development of the otic capsule. The otic capsule differs from other bones in showing very large numbers of bony canals as well as lamellar bone tissue. In the outer periosteal layer these canals are Volkmann's canals, which are similar to Haversian canals, but are multidirectional. Like the latter they are formed by canalisation of osteoblasts in the development lamellar bone. This outer layer is replenished throughout life by the production of further bone and canals from the surface periosteum. The middle layer is composed of bone and two types of canal: those formed from ossified cartilage and Volkmann's canals. This middle layer is replenished throughout life by the production of further bone and canals from the fissula ante fenestram. The bony intravestibular extension of the vestibular aqueduct is normally composed of large numbers of osteoblasts forming numerous Volkmann's canals and minicanals in a structure that we have named the "vestibulae arch". We found evidence that osteoblasts continually undergo slow apoptosis replenishing normally high levels of potassium in the endolymphatic duct. In Meniere's disease there is massive apoptosis of these cells with consequent denudation of Volkmann's canals and microcanals. The loss of cell nuclei may lead to a massive increase in potassium ions in the nearby endolymph and so to the severe endolymphatic hydrops characteristic of Meniere's disease. The new knowledge of otic capsule development has also clarified understanding of the histogenesis of otosclerosis. Otosclerosis is composed of compressed Volkmann's canals which form, like normal outer layer periosteal bone, in the otic capsule periosteum adjacent to the tensor tympani / processus cochleariformis complex and the canal of the internal carotid artery. The plaque invades downwards with darkly staining, poorly differentiated otosclerosis tissue constituting its invasive front. As it invades, earlier formed tissue undergoes differentiation so that at the origin of the plaque it is composed of highly differentiated lamellar bone and Volkmann's canals. It soon fixes the stapes footplate, but later invades cochlea and vestibule extensively, producing both sensorineural hearing loss and vestibular symptoms.The latter, as well as earlier symptoms of conductive hearing loss, should be realised as important features of otosclerosis.

Expression pattern of Olig gene family in the developing inner ears

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Olig gene family consists of Olig1 and Olig2 that were originally cloned as oligodendrocyte lineage-specific transcription factors and Olig3 that was found by homology search. Olig family genes are basic helix loop helix (bHLH) transcription factors that were important for cell fate specification in various organs of mammals. Other than oligodendrocyte differentiation, Olig1 regulates repair of demyelination in adult mammals and Olig2 regulates astrocyte and motoneuron development at the embryonic stage and glial scar formation after brain injury. Since many kinds of bHLH transcription factors, such as Hes1, Hes5, Hey2, Atoh1 and Neurogenin1, regulates inner ear development in various stages, we hypothesized that Olig family might have some important roles in inner ear development. To confirm this hypothesis, we checked expression pattern of Olig1, 2 and 3 in E10.5, E13.5 and E15.5 inner ears by in situ hybridization. All Olig genes were expressed in E10.5 otocyst epithelia and cochleo-vestibular ganglion (CVG) cells. Although Olig1 and Olig2 were expressed only in ventral portion of otocysts, Olig3 was expressed in all over the otocyst epithelia. Olig3 was strongly expressed both in inner ear epithelia and CVG cells even in E13.5 and E15.5 suggesting Olig3 might be important in the development of all inner ear epithelia. In contrast Olig2 was expressed in CVG cells and weakly in inner ear epithelia of E13.5 inner ears but not expressed in E15.5 inner ears. These results suggested that Olig2 might be involved in early stage of inner ear development. On E13.5 Olig1 was expressed only in CVG cells and not in inner ear epithelia but on E15.5 Olig1 became expressed in inner ear epithelia again, suggesting Olig1 affects inner ear development differently at early and later stages. In conclusion each Olig family gene has different roles in inner ear development.

Ush1c gene expression levels in the ear and eye suggest different roles for Ush1c in neurosensory organs in a new Ush1c knockout mouse

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Usher syndrome (USH) is the most common form of deaf-blindness in humans. Molecular characterization revealed that the USH gene products form a macromolecular protein network in hair cells of the inner ear and in photoreceptor cells of the retina via binding to PDZ domains in the scaffold protein harmonin encoded by the Ush1c gene in mice and humans. Although several mouse mutants for the Ush1c gene have been described, we generated a targeted null mutation Ush1c mouse model in which the first four exons of the Ush1c gene were replaced with a reporter gene. Here, we assessed the expression pattern of the reporter gene under control of Ush1c regulatory elements and characterized the phenotype of mice defective for Ush1c. These Ush1 knockout mice are deaf but do not recapitulate vision defects before 10 months of age. Our data show LacZ expression in multiple layers of the retina but in neither outer nor inner segments of the photoreceptor layers in mice bearing the knockout construct at 1-5 months of age. The fact that Ush1c expression is much higher in the ear than in the eye suggests a different role for Ush1c in ear function than in the eye and may explain why Ush1c mutant mice do not recapitulate vision defects.

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Impaired and derailed sensory neurons in the inner ear of Slitrk6-deficient mice

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Slitrks are type I transmembrane proteins that share conserved leucine-rich repeat domains similar to those in the secreted axonal guidance molecule Slit. They also show similarities to Ntrk neurotrophin receptors in their carboxy-termini, sharing a conserved tyrosine residue. Among 6 Slitrk family genes in mammals, Slitrk6 has a unique expression pattern, with strong expression in the sensory epithelia of the inner ear. We generated Slitrk6-knockout mice and investigated the development of their auditory and vestibular sensory organs. Slitrk6-deficient mice showed pronounced reduction in the cochlear innervation. In the vestibule, the innervation to the posterior crista was often lost, reduced, or sometimes misguided. These defects were accompanied by the loss of neurons in the spiral and vestibular ganglia. Cochlear sensory epithelia from Slitrk6-knockout mice have reduced ability in promoting neurite outgrowth of spiral ganglion neurons. Indeed the Slitrk6-deficient inner ear showed a mild but significant decrease in the expression of Bdnf and Ntf3, both of which are essential for the innervation and survival of sensory neurons. In addition, the expression of Ntrk receptors, including their phosphorylated forms was decreased in Slitrk6-knockout cochlea. These results suggest that Slitrk6 promotes innervation and survival of inner ear sensory neurons by regulating the expression of trophic and/or tropic factors including neurotrophins from sensory epithelia.

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Tmprss3, a serine protease deficient in human DFNB8/10 deafness, is critical for the hair cell survival at the onset of hearing

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Deafness is the most common sensory defect in human with one in every 1000 newborns affected. More than 60 % of deafness has a genetic origin. One of this deafness, DFNB8/10 is due to a defect in TMPRSS3, a type II serine protease. TMPRSS3 is a transmembrane protein with three extracellular domains. The LDLRA domain binds calcium and lipoprotein, the SRCR domain is involved in protein protein interaction and the serine protease domain is the catalytic one. To better understand the role of the protein in cochlear physiology, we have generated a mouse in which the tyrosine 260 was changed into a stop codon, thus deleting the major part of the serine protease domain. Offspring from heterozygote mating were tested for cochlear function by auditory brainstem response and balance function by rotarod and behavioural tests. Wild type and heterozygous mice have normal hearing thresholds out to 5 months of age whereas Tmprss3Y260X mutant mice are completely deaf. Concerning the vestibular system, Tmprss3Y260X mutant mice fall more quickly and display abnormal balance behaviour. Histological investigation showed the cochlear and saccular hair cells have degenerated. Scanning electron microscopy analysis during development demonstrated that cochlear hair cells develop normally until P12, then degenerate massively and rapidly following the well know base to apex gradient. In the saccule, hair cells begin to degenerate after P27 but the degeneration is less rapid and drastic. Our results show that Tmprss3 is essential for the survival of the cochlear and saccular hair cells. This animal model will allow deciphering the molecular mechanisms underlying DFNB8/10 deafness.

The expression pattern and the function of Septins in the mouse cochlea

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Septins are a family of cytoskeleton proteins which were identified in yeast as temperature sensitive mutants of cytokinesis. With the exception of plants, septins are well conserved in eukaryotic species. To date fourteen septin genes have been identified in mammals. Septins represent a fourth filamentous system after actin, intermediate filament and tublin because septins can form higher order filaments and scaffolds by heteromeric assembly and interact with other proteins including actin and tubulin and with components of the plasma membrane. To date, it has been reported that the septins are polymerizing scaffold proteins involved in cytoskeletal organization in mitosis, exocytosis and other cellular processes. In terms of morphology, cochlea contains many kinds of cells, some of which have complicated structure with polarity. Hair cells have hair bundles on their apical side. Supporting cells also contain bundles of supporting filaments extend to the apices of the hair cells. Lateral wall cells have many kinds of channels. To construct these complicated structures, scaffold proteins should be needed. In this study we examined the distribution of two subtypes of septins (septin4/5) in the adult mouse cochlea to investigate the relationship between the complicated structures of the cochlea and scaffold proteins, Septins. We also examined the auditory function of sept4 null mice In the immunohistochemical study, Sept4/5was detected below outer hair cells and basilar membrane in a fiber-like pattern in adult mice, but the expression pattern of Sept4/5 did not overlap with that of β3-tublin. So we concluded that Sept4/5 was expressed in Deiters' cells. However the expression pattern of Sept5 was not exactly the same as that of Sept4. While the filaments stained by Sept4 extended to the apices of the outer hair cells, the filaments stained by Sept5 extend only to the basal side of the outer hair cells. It was reported that Deiters' cells contain filaments some of which extend to the supporting cup and others to the apices of the outer hair cells. So the latter may not correspond to the filaments of Sept5 but Sept4. In addition, Sept5 was detected in the basal side of the inner and outer hair cells in a dot like pattern, and the expression pattern of Sept5 in the basal side of the hair cells overlaped with that of synaptophysin which was reported to be expressed in the presynaptic vesicle of the efferent nerve in the cochlea. It means that Sept5 is also expressed in the presynaptic vesicle of the efferent nerve in the cochlea. We also analyzed the morphology and function of the cochlea of Sept4 null mice. In ABR measurement, we observed that there was no difference in the auditory function between Sept4 null mice and Sept4 wild type mice. In immunohistochemical study we observed that Sept5 was expressed in the Deiters' cells in the same pattern as Sept4 in the Sept4 wild type mice in addition to their original expression pattern in the presynaptic vesicle of the efferent nerves. Considering these things together, we conclude that Sept5 compensates Sept4 defect in Sept4 null mice, and it is possible because Sept5 are most similar to Sept4 among Septins in the phylogenetic relationships.

Contribution of IL6 and STAT3 to otoprotection

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Background: Ototoxicity is a common cause of hearing loss. Recent reports implicated a significant role of proinflammatory cytokines (IL1-beta, IL6 and TNF-alpha) in the ototoxicity induced by cisplatin. The effects of IL1-beta and TNF-alpha on the auditory epithelium were previously studied. Here, we explored the effect of exogenous recombinant IL6 (rIL6) on the cochlea. Results: As an experimental model, we used the explanted organs of Corti (OCs) dissected from Wistar rats (p3-p5). First, by means of RT-PCR and immunofluorescence we positively confirmed the expression of IL6 receptor in the OC. Second, using immunoblotting and confocal microscopy we demonstrated that STAT3, which is an essential component of IL6 signaling pathway, is expressed by the auditory hair cells. We also found that the addition of rIL6 to the OC explant cultures induces phosporylation of STAT3 on tyrosine residue. Next, to determine the effect of IL6 on the viability of auditory epithelium, we cultured the OC explants with the rIL6 (0.3, 3, 30 and 90 ng/ml). We found no changes in number or in morphology of the auditory hair cells. Subsequent experiments demonstrated that the addition of rIL6 (30 ng/ml) to OC explants cultured with cisplatin (15 μM) does not exacerbate the loss of hair cells, as compared to cisplatin-treated controls. In contrast, more auditory hair cells have survived in the presence of IL6. The IL6-induced cytoprotection was observed only in the apical part of the OC and only in the inner hair cells. Conclusion: Taken together, our results suggest that IL6 signals in the rat OC. Further, IL6-induced signaling pathway may have otoprotective qualities when used together with cisplatin in vitro.

Trauma/inflammatory cascade-induced hair cell and hearing losses can be prevented by polymer- eluted dexamethasone

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Many different types of trauma to the cochlea (e.g. sound trauma) induce the loss of hair cell and hearing by initiating an inflammatory cascade that generates reactive oxygen species (ROS) and triggers the apoptosis of ROS-damaged hair cells (HCs). Local delivery of Dexamethasone (DXM) to the cochlea allows for corticosteroid therapy of cochlear tissues without serious side-effects.

P-3 Organ of Corti (OC) explants and TNF-alpha (an inflammation-related cytokine) challenged OC explants with and without polymer-eluted DXM. Inhibitors of glucocorticoid receptors and NFkB. Real time RT-PCR for an inflammatory mediator gene, anti- and pro-apoptosis-related genes. ELISA and immunostaining for phosphorylated NFkB. DXMb-eluting cochlear implant electrodes tested in an animal model of electrode insertion trauma (EIT)-elevation of pure tone thresholds and auditory HC loss. The control and experimental groups were: 1) EIT + Silicone electrode blank (SEB); 2) EIT + Silicone electrode blank, SIBS [poly(styrene-b-isobutylene-b-styrene)] coated (SEB+SIBS); 3) EIT + Silicone electrode blank, SIBS/DXM coated (SEB+SIBS/DXM); and 4) unoperated contra-lateral control ears.

In vitro: Polymer-eluted DXM protects HCs in TNF-alpha challenged OC explants against inflammatory-cytokine-induced apoptosis. Both TNF-alpha and TNF-alpha + DXM OC cultures show increased levels of NFkB protein compared to control cultures. Blocking NFkB signaling in TNF-alpha+ DXM treated OC explants prevents the otoprotective effects of DXM resulting in a cytokine-induced loss of the HCs. Gene expression studies show that TNF-alpha exposure upregulates TNF receptor 1 and Bax expression while down regulating the expression of both Bcl-2 and Bcl-xl promoting apoptosis of HCs. Treatment of either TNF-alpha challenged or naïve OC explants with DXM resulted to a down regulation of TNFR1 and Bax and an up regulation of Bcl-2 and Bcl-xl promoting HC survival. Blocking NFkB signaling in the TNF-alpha challenged, DXM treated OC explants prevented all DXM-initiated changes in gene expression that promote HC survival. Blocking the glucorticoid receptor also prevented DMX-initiated gene expression changes that promoteed HC survival. In vivo: EIT caused an initial increase in thresholds (i.e. temporary threshold shift; TTS) of auditory evoked brain stem responses (ABRs) to pure tone stimuli (i.e. 0.5 to 16 kHz) followed by additional increases in threshold over the next 2 weeks with these losses becoming permanent threshold shifts (PTS) at 1 month post-EIT. The SEB and SEB+SIBS groups of animals followed the above pattern of an initial TTS followed by a PTS at 1 month. The SEB+SIBS/DXM group of animals had an initial TTS followed by a near complete recovery of their initial pre-EIT threshold levels with no PTS at 1 month.

Polymer released DXM is as effective as locally applied DXM in the protecting of HCs from inflammatory cytokine-induced cell death. Polymer-eluted DXM signals through activation of NFkB, regulation of an inflammation-related gene, and pro- and anti- apoptosis members of the Bcl-2 family of genes. Dexamethasone eluted from a SIBS/DXM coated SEB prevents the initial EIT-related TTS from increasing and becoming permanent, i.e. PTS.

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Long-term antioxidant diet changes cochlear antioxidant capacity but not age-related hearing loss

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Oxidant stress appears to be a common feature associated with several inner ear pathologies. This has been well established for noise- and drug-induced hearing loss, and we have recently shown an oxidative imbalance in the cochlea of aging CBA/J mice. In the case of noise trauma and drug ototoxicity, hair cell loss and its functional consequences can be attenuated by antioxidant supplementation. Similar palliative treatments have been applied to alleviate age-related hearing loss but have yielded mixed results. We report here on a long-term feeding study to assess the influence of antioxidants on redox status and pathology in the aging cochlea. Female CBA/J mice were placed on a diet with supplementation of vitamins A, C and E, plus L-carnitine and lipoic acid. After eight weeks, the antioxidant capacity of the cochlea had significantly increased, validating the efficacy of the diet. The feeding regimens for the aging study began at an age of 9 months and two groups (regular diet and antioxidant supplementation) had well matched ABR thresholds at 4, 12, 24 and 48 kHz. Subsequent ABR measurements were taken at regular intervals and the animals were followed until an age of 24 months. By 18 months, thresholds had increased somewhat at all frequencies but no differences were apparent between the two groups. At 24 months of age, threshold shifts of ~20 dB had developed at all frequencies but again there was no difference between the groups (n = 37 each). Hair cell counts confirmed the lack of a protective effect of the antioxidant diet. Supported by program project grant AG025164 from the National Institute on Aging, National Institutes of Health.

Adenosine kinase inhibition in the cochlea delays the onset of age-related hearing loss in C57BL/6J mice

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Age-related hearing loss (ARHL), or presbyacusis, is the most common sensory deficit in human population. As the prevalence of hearing impairment increases with an aging population, there is a looming prospect of a deafness epidemic in the near future. This demands novel treatment strategies that would target the principal mechanisms of ARHL. Multiple mechanisms have been proposed for the age-related cochlear degeneration, and it appears that both genetic and environmental factors (oxidative stress) play a role. Inbred mice often show early onset ARHL, and the C57BL/6J mouse is the most established model of ARHL exhibiting behavioural and functional changes similar to those in the aging human ear. This mouse shows the onset of hearing loss at the age of three to six months and has a nearly complete hearing loss by the age of twelve months. This study was undertaken to determine the role of adenosine signalling in the development of ARHL. We and others have shown that the adenosine signalling system in the cochlea has an important role in cochlear protection from oxidative stress. Adenosine signalling is known to decline in the aging brain, and a similar process has been postulated to occur in the aging cochlea. Our study shows that adenosine A2A receptors and adenosine kinase are down-regulated in 9-month-old C57BL/6J mice, but their expression levels remain unaltered in CBA/J mice that maintain good hearing at old age. To compensate for the deficit in adenosine signalling in C57BL/6J mice, we have targeted adenosine kinase (ADK), the key enzyme in adenosine metabolism in the cochlea. Pharmacological inhibition of ADK leads to increased adenosine levels in cochlear fluids. The treatment was initiated with the selective ADK inhibitor ABT-702 (1.5 mg/kg intraperitoneally) in C57BL/6J mice at the age of three or six months. Treatment was maintained twice a week until the age of nine months and hearing thresholds were evaluated monthly using auditory brainstem responses (ABR). At the age of nine months, both groups treated with ABT-702 showed lower ABR threshold shifts (5-17 dB for auditory clicks and pure tone frequencies) compared to control animals receiving the vehicle solution. The better hearing of the ABT-702-treated mice was supported by increased survival of hair cells and reduced markers of oxidative stress (nitrotyrosine immunohistochemistry) and apoptosis (TUNEL staining) in the cochleae. This study provides the first evidence that the amplification of adenosine signalling in the cochlea can mitigate ARHL. The balanced activation of A1 and A2A receptors resulting from pharmacological inhibition of ADK is likely required for the survival and proper functioning of critical tissues in the aging cochlea. We postulate that increasing endogenous adenosine levels in the aging cochlea has multiple benefits which minimise the risk of oxidative stress, apoptosis and hearing loss in senescence. ADK targeting is thus emerging as attractive strategy for therapeutic management of ARHL.

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Effect of chloride and membrane holding potential on OHCs: simultaneous measures of electromotility and nonlinear capacitance

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Mammalian outer hair cell (OHC) electro-motility is believed to ultimately arise from conformational changes of the lateral membrane protein prestin. Under whole cell patch clamp, the conformation of prestin is monitored by its signature voltage-dependent nonlinear capacitance (NLC). A number of factors influence NLC, and shift prestin's voltage response profile. Both intracellular Cl and membrane holding potential (pre-pulse effect) alter NLC, shifting its voltage at peak capacitance (Vh) in the hyperpolarizing or depolarizing direction. In this study, we combine patch clamp and video imaging to record simultaneously OHC electromotility and NLC. As expected, we find that at 140 mM intracellular Cl concentration, there is an excellent match between Vh of NLC and motility. When intracellular Cl concentration is changed to more physiological levels, 10 or 1 mM, there is a disparity between each Vh. The Vh of motility is more depolarized than that of NLC. We find that the rate and direction of voltage stimulation, as well as turgor pressure, can influence the magnitude of this disparity. These data suggest that intracellular Cl modulates the coupling between prestin's conformational change and the whole cell response.

Mechanotransducer currents and resting potentials in cochlear outer hair cells

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Mechanotransducer (MT) currents in cochlear hair cells display fast and full Ca^{2+} driven adaptation making them relatively insensitive to small maintained mechanical perturbations. The fraction of the MT activated at rest is therefore determined by the properties of adaptation, which we hypothesize contributes to a standing inward current that depolarizes the resting potential. To test this hypothesis, we have measured MT currents in outer hair cells (OHCs) from isolated cochlear turns of postnatal day 7-12 (P7 – P12) rodents in response to hair bundle deflection. OHCs were patch clamped with electrodes filled with a K^+ based intracellular solution including 1 mM BAPTA, equivalent to the native endogenous calcium buffer. Exchanging the solution bathing the hair bundle from one containing perilymphatic (1.3 mM) Ca^{2+} to one resembling endolymph (0.02 mM Ca^{2+}) doubled the peak MT current and substantially increased the fraction of MT current activated at rest due to a negative shift in the activation relationship. The peak MT current in 0.02 mM Ca^{2+} was 1.19 +/- 0.05 nA (n = 5; gerbil, CF = 350 Hz) and 1.33 +/- 0.07 nA (rat, n = 5; CF = 4 kHz). The fraction of current activated at rest was 0.43 +/- 0.04 (gerbil) and 0.40 +/- 0.04 (rat). In perilymph (1.3 mM) Ca^{2+}, the fraction of current activated at rest was about 0.07 in both rodents. The increased fraction of current activated at rest generated a standing inward current of 0.51 +/- 0.05 nA and 0.54 +/-0.5 nA in gerbil and rat respectively. This standing current depolarized the resting potential from -50 +/- 2 mV (n = 5; 1.3 mM Ca^{2+}) to -34 +/- 4 mV (0.02 mM Ca^{2+}). Confirmation that the depolarization in 0.02 mM was due to opening of MT channels is that it was blocked by addition of 0.2 mM dihydrostreptomycin which hyperpolarized the OHC to -54 +/- 2 mV. Maximum receptor potentials recorded in OHCs were 40 to 50 mV and with addition of an 80 mV endolymphatic potential exceeded 100 mV peak-to-peak. Although not examined, the endolymphatic potential will augment the standing MT current in vivo, and hence further depolarize the OHCs. We suggest that OHCs in vivo have a depolarized resting potential of positive to -40 mV. This conclusion differs from previous findings using sharp electrode recordings in vivo where OHC resting potentials were measured to be negative to -70 mV (Dallos, 1985; Cody and Russell, 1987). Such a negative resting potential is likely to reflect a compromised MT apparatus (reduced or absent resting inward standing current), as suggested by the small receptor potentials of less than 15 mV peak to peak measured in these cells. Supported by grants from National Institutes on Deafness and other Communication Disorders (RF) and Wellcome Trust (WM).

Analysis of transmembrane structure of prestin by single molecule force spectroscopy

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Outer hair cells (OHCs) longitudinally elongate and contract in synchronization with change in the membrane potential. This motility is thought to be based on the voltage-dependent conformational changes of the motor protein prestin densely embedded in the plasma membrane of OHCs. Prestin consists of 744 amino acids with a molecular weight of about 81.4 kDa. In a series of attempts to clarify its membrane topology, hydrophobicity analysis in conjunction with the prediction of the conserved phosphorylation site has suggested that prestin is a 10 or 12 transmembrane protein with cytoplasmic N- and C-termini. Regarding the size of prestin, prestin has recently been reported to form oligomers, presumably about 10 nm in diameter. Although extensive researches and experiments have been conducted regarding the structure of prestin, it has not yet been clarified. As the structure of prestin is closely related to its function, further research on prestin at the molecular level is required to elucidate its function. In the present study, a simple and direct single-molecule technique producing detailed information about the structure of prestin molecules using an atomic force microscope (AFM) was developed, so-called single molecule force spectroscopy (SMFS). This method can be applied to samples in liquid, allowing biological structures to be observed in a close-to-native environment. The C-terminus of prestin was tagged with a receptor peptide Avi-tag. This tag was then enzymatically biotinylated by a biotin ligase so that prestin connects with a streptavidin-coated AFM cantilever via biotin-streptavidin binding. The Avi-tagged prestin was pulled out from the plasma membrane and force curves were obtained. Obtained force curves suggested the existence of 12 transmembrane domains of prestin. Helix 2 of prestin had weaker unbinding force than the other helices of prestin, suggesting the flexibility of this domain, possibly relating the conformational change of prestin.

Identification of the electromotility motif in prestin, the motor protein of cochlear outer hair cells

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Cochlear outer hair cells (OHCs) are capable of altering their length in response to transmembrane voltage change. This so-called electromotility is the result of conformational changes of membrane-bound protein prestin. Prestin-based OHC motility is believed to be responsible for cochlear amplification that contributes to the exquisite frequency selectivity and sensitivity seen in mammalian hearing. Prestin belongs to a distinct anion transporter family called solute carrier protein 26A, or SLC26A. However, Prestin is unique in this family, functioning as a voltage-dependent motor protein manifested by two hallmarks, nonlinear capacitance and motility. Recent evidence suggests that prestin orthologs from zebrafish and chicken are divalent/chloride anion exchangers/transporters with no motor function. We identified a segment of 11 residues in mammalian prestin that is well conserved among mammalian species but highly variable among non-mammalian orthologs and SLC26A paralogs. To determine whether this region represents the minimal essential motif for the motor function, we utilized a chimeric approach by swapping corresponding residues from the zebrafish and chicken with those from the gerbil. Motility and nonlinear capacitance were measured from chimeric prestin-transfected human kidney embryonic cells using voltage-clamp technique and photodiode-based displacement measurement system. We observed a gain of motor function with both hallmarks in the chimeric prestin. Our results show, for the first time, that the substitution of a span of 11 amino acids confers the electrogenic anion transporters of zebrafish and chicken prestins with motor function. Therefore, this segment represents the minimal essential motif for the motor.

Translocation of prestin having mutation in the GTSRH sequence by salicylate

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The motor protein prestin in the plasma membrane of cochlear outer hair cells is thought to be an origin of their electromotility. Several characteristics of prestin have been clarified by introduction of mutations into prestin. In the present study, the aim was to investigate whether or not salicylate has the ability to promote the plasma membrane expression of prestin mutants accumulated in the cytoplasm. Six prestin mutants, namely, G127A, T128A, S129A, R130A, H131A and S129T, were created. These mutants were engineered to be expressed in HEK293 cells by transfection and effects of salicylate on prestin were then investigated by immunofluorescence staining and by whole-cell patch-clamp. When the cells were cultured without salicylate, immunofluorescence staining showed that all prestin mutants were accumulated in the cytoplasm. The patch-clamp recording indicated that H131A and S129T did not show nonlinear capacitance (NLC), which reflects the amount of functional prestin in the plasma membrane, and that the other four mutants showed NLC significantly smaller than that of wild-type (WT) prestin. On the other hand, when 10 mM salicylate was used, immunofluorescence staining suggested that the plasma membrane expression of all prestin mutants was recovered. The plasma membrane expression of G127A and R130A was recovered to the WT prestin level. By the patch-clamp method, NLC in G127A, T127A, S129A and R130A were shown to statistically increase, although H131A and S129T did not exhibit NLC. Especially, NLC of G127A and that of R130A recovered to the WT prestin level by salicylate. The results suggest that the prestin mutants were misfolded in the cytoplasm and that salicylate has the ability to induce mutants' correct folding, promoting their transport to the plasma membrane, which led to the recovery of NLC.

Self-tuning and coupling between hair cells of the inner ear

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Theoretical models have proposed that a hair cell constitutes a nonlinear system with an internal feedback mechanism that can drive it across the Hopf bifurcation and into an unstable regime. We will present latest results on the self-tuning in hair cells in response to external mechanical input. Secondly, we have developped techniques that allow us to track movements of multiple hair bundles in parallel. With extracellular coupling elements left intact, the hair cells exhibit a significant degree of phase-locking, over the relevant physiological range of stimulus. We demonstrate that this inter-cell coupling plays an important role in shaping the response of the system.

Somatostatin receptor-1 and -2 in the mammalian cochlea

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Little is known about expression and function of the somatostatinergic system in the mammalian cochlea. In this study, we analyzed the expression of somatostatin receptor 1 (SST1), somatostatin receptor 2 (SST2) and somatostatin in the immature and mature mammalian cochlea using immunohistochemistry. We demonstrate that the somatostatin receptors SST1 and SST2 are expressed in outer and inner hair cells (HCs) of the organ of Corti (OC) as well as in defined supporting cells. In addition, we show that functional maturation of the OC involves changes in the expression patterns of these two receptors. Interestingly, somatostatin itself is not expressed in the mammalian cochlea, suggesting that somatostatin reaches its receptors either trough the blood-labyrinthine barrier from the systemic circulation or via the endolymphatic duct from the endolymphatic sac. In order to learn more about the regulation of SST1 and SST2 receptors, we used mice with either a deletion of SST1 or SST2. We demonstrate that in SST1 knock-out mice, SST2 is expressed in outer HCs and Deiter's cells, but not in pillar cells and inner HCs as compared to wild-type mice. In contrast, in SST2 knock-out mice, the expression pattern of SST1 receptor is not altered compared to wild-type mice. These findings provide evidence of a compensatory regulation in the mammalian cochlea as a consequence of a distinct somatostatin receptor deletion. Since compensatory events can be observed after SST1 deletion but not after SST2 deletion, this indicates that the compensatory event is receptor subtype specific.

A new approach for semi-automatic segmentation of the cochlea from CT images

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The segmentation of the cochlear fluid chambers (scalae) is difficult due to their complicated geometry. To perform planar segmentation on the cochlea efficiently, it is necessary to reslice the image stack to ensure that the segmented cross-section is always transversal. Additional obstacles on tomography images are the weakness of soft structure representation and digital noise. We present a new 3-D approach for segmenting cochlear scalae from micro computer tomography (μCT) images in presence of noise semi-automatically. An elongated structure like a cochlear scala is characterized by its central path which is the total of center points of all cross-sections. Our segmentation method performs a prediction of the course of the central path using Kalman filter. This is done to find the transversal image plane which is perpendicular to the central path. The segmentation of the scala cross-section is being performed using the active contour method. The centers of the resulting cross-section estimation are being computed and used for further central path prediction and sectioning. In this way, the algorithm iteratively propagates through the desired structure. For our experiments, we used isolated human and guinea pig cochleae and stained them with iodine and osmium tetroxide, respectively. After preparation and staining, the specimens were recorded with a μCT device. Prior to segmentation, the image stack was noise-reduced with a bandpass filter and anisotropic diffusion algorithm. Using our semi-automatic segmentation approach, we successfully labelled all three scalae of the guinea pig cochlea and the scala tympani of the human cochlea from the basal to the apical end. The method has been implemented in our free software “IPTools“ which is downloadable at http://www.tu-dresden.de/medkhno/sites/wissenschaft/mittelohr/index.htm. Our approach is not limited to cochlea as object of investigation. It is suitable to any elongated biological object with a smoothly changing cross-section, e.g. cells or intracellular structures. It can be also used with image stacks obtained from other techniques like LSM, MRT or TEM tomography.

Glutamate agonist causes irreversible degeneration of inner hair cells

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Glutamate neurotoxicity in cochlear hair cells was investigated by administering the glutamate agonist α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) into the scala tympani of Mongolian gerbils. AMPA administration caused the formation of large numbers of vacuoles in the inner hair cells (IHCs) and dendritic terminals. The number of degenerate hair cells was counted using Rhodamine-phalloidin and Hoechst 33342 staining. The administration of 50 μM AMPA caused reversible elevation of the auditory brainstem response (ABR) threshold without loss of IHCs. By contrast, 200 μM AMPA induced a substantial elevation of the ABR threshold with the characteristic disappearance of IHCs. As cochlear ischemia involves excessive glutamate release, these results suggest that an elevated glutamate level in the cochlea is responsible for the progressive IHC death related to ischemic injury.

A re-examination of the striated organelle in vestibular endorgans

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The striated organelle (SO) is a structure located in the subcuticular region of inner ear hair cells, consisting of alternating thick and thin filaments (Friedman et al., 1963; Ross, 1983). Although present in cochlear inner hair cells and both types of vestibular hair cells, the SO is particularly well-developed in type I vestibular hair cells. It is shaped like an inverted, open-ended cone that contacts the cell membrane along its entire circumference and is separated from the cuticular plate by a layer of large mitochondria. In other hair cells, it is less extensive and appears to be free-floating. We studied its structure in rat and chinchilla hair cells with electron microscope (EM) tomography. In three-dimensional reconstructions, we have found that it is connected to at least some actin rootlets and is associated with microtubules, mitochondria and smooth endoplasmic reticulum. Actin rootlets contact the hair cell membrane near the SO, opposite the kinocilium. The subcuticular mitochondria are 5X larger in volume and 2X larger in surface area than those in the rest of the hair cell or those in type II hair cells. Our attempts to determine its protein composition have so far included immunohistochemical approaches. Confocal immunofluorescence places an actin-binding protein, alpha 2-fodrin (brain spectrin), where the SO contacts the hair cell membrane. EM immunogold studies place fodrin in the thick filaments. Contact with the rootlets suggests that the SO might regulate hair-bundle stiffness, while its association with the cell membrane suggests that the SO may help form the constricted neck characteristic of type I hair cells. We are currently using isolated hair cells to investigate the function of the striated organelle.

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Morphological change of the cupula and its effect on the semicircular canal activity

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Objectives: It is expected that the cupula sustains morphological changes, such as shrinkage or deformity under some conditions. This study was aimed to examine the effect of half-sized cupula on the semicircular canal nerve potential induced by mechanical endolymphatic flow and thermal stimulus. Method: Bull frogs were used. They were subjected to gentamicin (GM) injection into the perilymphatic cistern, or mechanically rupturing the membranous labyrinth. The cupulae were observed at 7 or 14 days after GM injection by stereoscopic microscope. The ampullae were fixed, and the sensory cells were assessed using a scanning electron microscope (SEM). The correlation between the cupula and SE changes was evaluated. For mechanical rupturing, a fine needle was inserted into the inner ear so that it penetrates directly the center of the saccular otoconia. After 1, or 2 weeks, change of the cupula was observed and semicircular canal nerve compound action potentials (CAPs) in response to mechanical endolymphatic flow were recorded. The cupula was removed from the crista and was sectioned in half using fine scissors to simulate shrunken cupula,. The half cupula was replaced on the crista and CAPs were recorded. Also, CAPs were recorded under thermal (cool) stimulus to simulate caloric test. Results: In over half of the cupulae of the 7- and 14-day GM injection groups, shrinkage, or deformity of the cupula was observed. The shrinkage started from the margins, including the apex. In about 50% of the total cases, the degree of cupula and sensory cell change did not correlate, indicating that the cupula alone can sustain changes without sensory cell damage. Mechanical rupturing of the membranous labyrinth induced various degrees of cupula changes, such as shrinkage, deformity and volume enlargement. CAPs could be recorded even in the markedly damaged cupula. With half-sized cupula, the CAP became smaller than the normal sized cupula under slow stimulus. With half-sized cupula, CAP was not recorded under thermal stimulus. Discussion and conclusion: There are many studies on the effects of ototoxic agents on the inner ear. However, most of them dealt with sensory cells, and there are few studies on the appendicular part of the sensory organ, such as cupula. In this study, the cupula shows various changes after GM injection, with or without damage of the sensory cells. Cupula shrinkage started from its periphery, leaving a space between it and the ampullary wall. In this condition, the endolymphatic flow effect decreases because the endolymph passes through the gap, thus possibly resulting in canal paralysis (CP) of the caloric response even if the sensory cells function is normal. This was confirmed by decreased CAP under slow mechanical endolymphatic flow stimulus and thermal stimulus in half-sized cupula preparation. Under these conditions, the shrunken cupula potentially behaves as a floppy cupula with mobile top part, thus resulting in more intense nystagmus and dizziness. If the cupula change develops during or after the inner ear disorder, such as vestibular neuritis or Meniere's disease, it might lead to persistent positional vertigo. We need to pay more attention to disorders of the cupula other than the sensory cells as a possible lesion of peripheral vertigo and mechanism of caloric CP.

Myelination and protein expression associated with human spiral ganglion neuron preservation

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We investigated the expression of myelin basic protein (MBP) in the human auditory nerve's myelin sheath and the satellite glia cells (SGCs) enwrapping spiral ganglion cell bodies using immunohistochemistry and laser confocal microscopy on cryostat sections. Parafin sections from patients with sensorineural hearing loss were also investigated regarding general morphology and vassculature. The dendrites and axons in the cryosections were found to express MBP while SGCs did not. Findings are consistent with TEM investigations showing lack of myelin around neural perikarya in man in opposite to most mammals studied so far. SGCs formed a more or less incessant honeycomb-like system expressing S-100 and Cx43. The glial network may partly integrate vasculature; an alliance also prominent in areas where neurons have lost dendrites. A satellite glial syncytium improve survival properties of ganglion cells following deafferentiation. and could also integrate neuronal signaling. Cx30 immunoreactivity were found in human SGNs. Its significance is not known

Voltage-dependent ion conductances in long-term cultures of rat spiral ganglion neurons

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Spiral Ganglion Neurons (SGNs) of the cochlea form a critical functional interface between the mechano-electrical transduction step in the inner ear and the higher-level sound processing in the central nervous system. As so, SGNs represent an important cellular target for the intervention and modulation of molecular processes underlying sound perception in pathological conditions associated with different forms of deafness. Cultured SGNs from early postnatal animals represent a potentially valuable in vitro tool to investigate excitability properties might be modulated by over-expression of specific ion channels. However, successful delivery of nucleic acids by means of standard transfection techniques requires long-term survival of such cultures and their proper maturation. Therefore, in contrast to previous electrophysiological examination of acutely isolated gerbil SGNs (10-18 hours in culture; Lin X, Hear Res 1997) we examined the ionic conductances in the long-term SGN cultures (20 days) derived from P4-P5 rat cochleae. Morphologically, in contrast to acutely-isolated SGNs, long-term cultured cells with a bipolar soma grow long processes, typical for properly regenerated neurons. Labelling with the anti-neurofilament antibody confirmed the neuronal identity. whole-cell patch-clamping analysis shows that they express all three ion conductances necessary for the firing of action potentials: Sodium, quickly inactivating, current was activated by depolarisation pulses from a pre-pulse of -100 mV. Potassium, slowly inactivating, currents were elicited by depolarisation pulses and the hyperpolarization-activated current Ih by hyperpolarization pulses from a holding potential of -70 mV. The voltage-dependence of activation as well as the kinetics of such currents are typical OF adult, mature SGNs (Szabo ZS et al., Eur J Neurosci 2002). Based on such morphological and electrophysiological observations, long-term SGN cultures represent a valuable in vitro system to investigate the modulation of the ionic conductances underlying the excitability of mature, fully functional, spiral ganglion neurons.

Intrinsic determinants of enhanced mid-frequency sensitivity in murine spiral ganglion neurons

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Our studies of the intrinsic properties of auditory neurons in vitro have revealed a systematic variation along the tonotopic axis of the cochlea. Features such as onset time course, latency, and action potential duration show a monotonic gradient with fast firing features most prevalent in neurons that innervate high frequency cochlear regions. Interestingly, the distribution of neuronal firing threshold is distinctly different, exhibiting a non-monotonic, bell-shaped gradient such that neurons with the lowest thresholds are located toward the mid-apical innervation regions. This was surprising, because our expectation was that frequency sensitivity would be determined solely by middle ear and cochlear mechanics (Rosowski, J Acoust Soc Am, 1991, Ruggero and Temchin, Proc Natl Acad Sci USA, 2002) as well as synaptic mechanisms (Merchan-Perez and Liberman, J Comp Neurol, 1996, Meyer et al., Nat Neurosci, 2009). Yet firing thresholds in spiral ganglion neurons are apparently set independent of the hair cell input since our recordings were made from isolated neurons in vitro (Liu & Davis, J. Neurophysiol. 2007). To explore the issue of excitability, we also examined resting potential, a feature that in tandem with threshold would determine spiral ganglion neuron responsiveness to depolarization. We reasoned that neurons in the mid-apical cochlear regions might have less negative resting potentials that along with lowered thresholds would also reduce the depolarization required to elicit action potentials. Our prediction was confirmed; decreased threshold in the mid-apical cochlear region was indeed accompanied by an elevated resting potential (-68.70 ± 0.55 mV, n=24; -65.26 ± 0.66 mV, n=26; -65.54 ± 0.66 mV, n=23 for base, middle and apex neurons, respectively). Studies are currently underway to elucidate the determinants of the increased excitability in mid-apical spiral ganglion neurons. Our initial focus has been on individual classes of low-voltage activated ion channels such as the shaker-related potassium channels. Application of alpha-DTX (100nM), a specific blocker of these channels, produced both a substantial change in neuronal threshold (Δ = -25.73 ± 2.04 mV, n=11; -14.19 ± 1.28 mV, n=8; -14.86 ± 2.23 mV, n=7 for base, middle and apex neurons, respectively) as well as systematic alterations in resting potential (Δ = 9.95 ± 0.70 mV, n=4; 7.84 ± 0.89 mV, n=8; 7.21 ± 0.31 mV, n=8 for base, middle and apex neurons, respectively). Examination of another candidate current, Ih, which we blocked with CsCl (5 mM) revealed that this current also contributes to setting the resting potential but without a noticeable impact on threshold. Consistent with the above alpha-DTX experiments, resting potential changes were greater in the basal and apical neurons than in the middle neurons after CsCl application (Δ = -3.86 ± 0.86 mV, n=6; -2.74 ± 0.70 mV, n=8; -3.94 ± 0.83 mV, n=5 for base, middle and apex neurons, respectively). Future studies will assess involvement of other channel types, such as the KCNQ4 potassium channels. In summary, our investigation of spiral ganglion neuron physiology continues to reveal unexpected sophistication in membrane properties that control their responsiveness and response characteristics. These features are produced through precisely regulated ion channel gradients which ultimately generate the accurate neuronal codes necessary for perception of auditory stimuli.

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Effects of electrical stimulation on the acoustically evoked compound action potential

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The criteria for implantation with a cochlear prosthesis have been broadened and people with considerable residual low-frequency hearing receive an implant nowadays. This raises the issue of interaction of electric and acoustic stimuli in the same cochlea. We have investigated the effects of ipsilateral intracochlear electrical stimulation on the amplitude of the acoustically evoked compound action potential (CAP) in normal-hearing guinea pigs and animals with high frequency hearing loss. Effects of electrical stimulation on CAP amplitude were tested using a forward masking paradigm. Electric stimuli were delivered via a 1 mm platinum wire electrode inserted in the basal turn of the cochlea. The return stimulation electrode was placed extracochlearly on the basal turn of the cochlea. Electric stimuli consisted of trains of biphasic pulses 10 ms in duration. Current level was typically 800 μA. CAPs were evoked with acoustic tone bursts (8 or 12 ms duration). CAP recording was performed with an apical electrode. CAPs were evoked at various acoustic frequencies (0.5 to 16 kHz) and various acoustic levels. The effect of the interval between electric and acoustic stimulus (EAI) was also tested. Effects of the pulse rate on CAP amplitude was tested with pulse trains varying from 0.5 to 4 kHz (500 – 4000 pulses/sec). CAPs evoked with high-frequency tones were suppressed by electrical stimulation. This suppression was most pronounced at low sound levels. CAP suppression at high frequencies was clearly dependent on EAI and decreased at increasing EAIs. Recovery of high frequency-evoked CAPs was typically complete at EAIs as short as 2 milliseconds. We have strong indications that there is a bimodal mechanism behind CAP suppression by electrical stimulation. There seems to be a direct neural interaction based on refractoriness, but also suppression by mechanical events in the cochlea, based on electrophonics, i.e., a traveling wave evoked by the electrical pulses. The clinical implication is that our data indicate that basal (high-frequency) cochlear regions can be stimulated electrically without affecting low-frequency acoustical hearing. The finding that the CAP amplitude recovered to normal values within a few milliseconds after the electrical stimulus may be interesting for hybrid implant stimulation strategies.

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The transcriptional response of spiral ganglion neurons to deafferentation is partially ameliorated by acute electrical stimulation

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Hair cells are the primary but not sole source of trophic support to spiral ganglion neurons (SGNs) of the cochlea. In the absence of hair cells, SGNs have a tendency to apoptosis that may be mitigated by membrane depolarization and/or neurotrophins. A number of molecular and genetic mechanisms whereby membrane depolarization prevents apoptosis have been studied in our lab. The objective of the current study is to contextualize activity of neurotrophic and apoptotic pathways in the transcriptomes of SGNs of hearing and recently deafened rats and to investigate how activation of these pathways is altered by acute patterened electrical stimulation (ES) comparable to that provided by a cochlear implant (CI). The rat model is ideal for this investigation because its genome has been sequenced, rat SGN apoptosis has been studied extensively in our lab, and it is anatomically suitable for studies of ES. Gene expression microarrays were generated from spiral ganglia of P32 rats. Briefly, perinatally deafened rats were unilaterally implanted at P32 for 8 hours of acute ES (monopolar, biphasic, 100 Hz, amplitude 2x threshold). Microdissected spiral ganglia were split into apex and base. Contralateral cochleae were used as unstimulated operative controls. Age and litter-matched deafened and hearing controls were also assayed using expression arrays. RT-qPCR, western blot and in situ hybridization were used to follow up on genes of interest. 45 out of 544 genes that had significant (>2 fold) modulation after deafferentation were also reversed by electrical stimulation. Direct neurotrophin and apoptotic precursors were not significantly changed. Interestingly, deafferentation induced a decrease in the noncanonical Wnt pathway that was reversed by ES. Acute ES is able to reverse the effects of a small percentage (8.3%) of SGN transcriptional changes in response to deafferentation. Apoptotic genes are not directly modulated. Further work is needed to assess how chronic ES and/or exogenous neurotrophins affect SGN transcription.

The expansive relation between instantaneous rate of period histograms and instantaneous pressure of pure tones is retained at high stimulus levels

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The relation between instantaneous sound pressure p(t) of pure-tone stimuli and instantaneous spike rate I(t) in period histograms can be described by the exponential relation $I(t)=I0\exp(\alpha p(t))$ (Horst et al., 44th IEB-Workshop, p83, 2007) in the region between spontaneous activity I0 and rate threshold. This relation holds for low, medium and high-spontaneous rate fibers. The factor α describes the sensitivity of the nerve fiber: the higher the threshold, the smaller α. We investigated the relation between instantaneous rate and instantaneous pressure at stimulus levels where refractory effects play a role. We plotted these data as instantaneous input-output (IO) curves. For stimulus levels near threshold, the rate-level curve has an expansive behavior. In this region IO-curves concide for responses collected at different levels. For higher levels, where the rate-level curve is fairly linear, the IO curves fall more and more below the IO curves determined at lower levels. I.e. for a given instantaneous pressure the instantaneous rate is lower for the stronger stimulus. In addition to that the IO-curve shows hysteresis. We investigated the responses at high levels by plotting the IO-curves on logarithmic instantaneous rate (IR) vs. linear pressure (p) scale. This has the advantage of rendering a straight line if there is an exponential relation between p and IR. We found that for higher levels the IO-curve rather showed a decrease of slope α than a shift down of the IO-curve. In order to reduce the role of refractory effects, we studied the shape of the histograms and IO-curves if we only accepted spikes after a minimal interspike interval (Gray, 1967). By means of this procedure the IO-curves can be interpreted as representing “recovered probability”, i.e. probility of firing corrected for refractory effects. After application of this method, up to minimal ISIs of 32 ms, the slope of the IO-curves was retained and some of the hysteresis was still presented. Our data confirm that the IO-curves remain expansive at high levels, in contrast to the compressive behavior of rate-level curves.

Cochlear repair and hair cells regeneration after human pluripotent stem cell transplantation to nod-scid mice made deaf with kanamycin and intense noise

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We investigated the fate of human umbilical cord blood CD133+ hematopoietic stem cells (HSC) transplanted intravenously (IV) into irradiated nod-scid mice (genetically unable to reject these cells) made deaf by ototoxic treatment with kanamycin and/or intense noise, to verify whether HSC engraft the cochlea and contribute to inner ear restoration in vivo. We tested the presence of HLA-DQα1 by PCR , used for traceability of engrafted cells, finding PCR evidence that HSC migrated to various host tissues, including the organ of Corti in the cochlea. By histology, antibody and lectin staining analysis, we confirmed that HSC IV transplantation in mice previously damaged by ototoxic agents correlated with the repair process and stimulation ex novo of morphological recovery in the inner ear, while the cochlea of control oto-injured, non-transplanted mice remained seriously damaged. FISH analysis, to detect human genomic sequences in the nuclei of cells, also confirmed persistent engraftment of small numbers of chimaeric cells in various mouse tissues and organs (e.g. spleen, liver, kidney) including the inner ear, for up to 2 months following transplantation. Dual color FISH analysis, for detecting both human and mouse centromeric DNA sequences, also revealed small number of heterokaryons, probably derived from fusion of donor with endogenous cells, whose number decreased with time. Additionally, we investigated the effect of bone-marrow derived mesenchymal-like pluripotent stem cells transplanted IV to oto-injured mice and obtained comparable positive results; in contrast oto-damaged mice that were not transplanted remained irreversibly damaged . These observations offer the first evidence that transplanted stem cells migrating to the damaged inner ear may provide conditions for the resumption of the injured cochlea facilitating the regeneration of NE and hair cells, emerging as a potential strategy for inner ear rehabilitation.

An *in vitro* model of synapse degeneration and regeneration in the cochlea

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Inner hair cells (IHCs) and adjacent supporting cells produce NT-3, the principle neurotrophin in the postnatal organ of Corti (OC). Spiral ganglion neurons (SGNs) express both TrkB and TrkC so can respond to both BDNF and to NT-3. We used a cochlear explant culture from P6 rat pups consisting of a portion of the OC maintained intact with the corresponding portion of the spiral ganglion as an in vitro model to investigate the role of NT-3 in reinnervation of IHCs by SGNs. The normal innervation pattern, i.e., each type 1 SGN contacting a single IHC, is quantitatively preserved in vitro. Brief treatment with the glutamatergic agonist kainic acid (KA) results in loss of IHC -SGN synapses and degeneration of the distal ends of type 1 SGN peripheral processes, mimicking cochlear damage caused by excitotoxicity or noise in vivo. Reinnervation of IHCs occurs and regenerating processes remain restricted to the IHC row. However, the number of postsynaptic densities (PSDs) does not fully recover; not all processes regrow to the IHCs, and those that do regrow inappropriately innervate multiple IHCs. Addition of either NT-3 or BDNF increased process regrowth, increased the number of PSDs, and reduced multiple innervation of IHCs. Selective blockade of intrinsic NT-3 signaling with a TrkC-IgG fusion protein resulted in a profound reduction in SGN processes and PSDs on IHCs but blockade of BDNF signaling had no effect. TrkC-IgG even reduces the numbers of PSDs and of SGN peripheral processes in contact with IHCs in the presence of BDNF. That is, in the absence of endogenous NT-3 signaling, even exogenous BDNF can't improve reinnervation. These data indicate that endogenous NT-3 has a specific functional role, distinct from that of BDNF, in synaptogenesis in the OC. In addition to Trk-family receptor, neurotrophins bind the neurotrophin receptor p75NTR, although only unprocessed proneurotrophin forms bind with high affinity and are the physiological p75NTR ligands, just as the processed forms are the physiological Trk ligands. p75NTR is expressed in the neonatal rodent cochlea but only in inner pillar cells and is not normally expressed in the mature cochlea. However, p75NTR and a ligand, proBDNF, are upregulated in the OC following excitotoxic trauma. We investigated the role of p75NTR in synaptic regeneration in 4-6 day old rat and mouse organotypic cochlear cultures. p75NTR knockout mice have normal hearing and cochlear innervation but reinnervation after excitotoxic trauma is greatly deficient. We used 0.8 nM proNGF to selectively activate p75NTR but not TrkB or TrkC expressed on SGNs (TrkA is not expressed in the cochlea). ProNGF significantly increased reinnervation of IHCs after KA treatment, increasing the number of PSDs and SGN peripheral processes on IHCs. This is blocked by TrkC-IgG indicating that it is NT-3-dependent and, correspondingly, proNGF significantly increases NT-3 expression in the OC. In summary, we have developed an in vitro system to investigating the effects of excitoxicity on IHC-SGN synapses, recovery, and reinnervation. This is crucial to developing therapeutic strategies to recovery from noise damage to SGNs. We show NT-3 is the key endogenous neurotrophin for maintaining synapses IHC-SGN synapses. NT-3 levels decline after excitotoxic trauma but a novel mechanism is recruited to promote partial recovery: p75NTR and a ligand are upregulated in the OC, cause increased NT-3 expression and improved reinnervation.

A role for Hensen cells in the resolution of inflammatory responses in the cochlea

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It is now recognized that endogenous factors released by specific cells counter-regulate inflammation and promote resolution, reverting the cells and tissues back to a non-inflammatory phenotype. Thus, the resolution phase of the inflammatory response should be considered an active rather than a passive process. The mechanism used by the cochlea to complete the resolution phase of the inflammatory response is still unknown, except by the clinically exploited fact that it can be stimulated by glucocorticoids. We used microscopy, immunocytochemical and microfluidic techniques to elucidate the effect of dexamethasone, hydrocortisone and prednisolone on the cellular and intracellular distribution of annexin A1 (ANXA1), a pro-resolution protein, in the cochlea and isolated cochlear cells of guinea pigs. In addition, we used cochlear Hensen cells and HEI-OC1 cells to investigate in vitro the glucocorticoid-activated mechanism of ANXA1 release. Our results indicate that, although all the cells lining the scala media express ANXA1, it is mostly concentrated inside the lipid droplets within Hensen cells. We also found evidence that glucocorticoids activate a myosin 2C-mediated mechanism that drives ANXA1 to the apical region of the cells, where ANXA1 is released to the external milieu. ANXA1 released by guinea pig Hensen cells, in turn, was shown to inhibit migration of polymorphonuclear (PMN) leukocytes in vitro. Based on these results, we propose that ANXA1 would be responsible for inhibiting migration of PMN and other leukocytes into the scala media, facilitating the resolution phase of inflammatory responses in the mammalian cochlea and thus protecting the organ of Corti against potential disruption of the tight junctions responsible for maintaining the endocochlear potential.

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Matrix metalloproteinases -2 and -9 in the cochlea: expression and activity after aminoglycoside exposition

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Introduction: The different cell types in the cochlea are embedded in a highly organized extracellular matrix (ECM). Homeostasis is maintained within the ECM by regulating the turnover of matrix composition, in which the matrix metalloproteinases (MMPs), a family of zinc-dependant proteases, play a major role. These proteases have been well studied in the retina and the brain, remarking their importance in neuronal cell survival and death (Chintala et al., 2006; Candelario-Jalil et al., 2009). Do to common characteristics shared by the neuroepithelia of the eye and the inner ear, we focused on the localization and function of MMP-2 and MMP-9 in the cochlea, determining their expression and activity under normal conditions and after aminoglycoside exposition. Materials and Methods: Expression of MMP-2 and MMP-9 in 5-day-old Wistar rat cochleas was analyzed by RT-PCR, real-time PCR and Western blot. In C57BL/6 adult mice the presence of MMP-2 and MMP-9 within the cochlea was localized by immunohistochemistry. Expression levels of MMP-2 and MMP-9 on the mRNA and the protein levels of organotypic cultures of OCs exposed to 0.25 mM gentamicin were compared to non-treated control explants. Furthermore, auditory hair cells loss of organotypic cultures incubated with and without the presence of 0.25 mM gentamicin and/or 50 µM MMP Inhibitor was analyzed. Results: We observed that MMP-2 and MMP-9 proteins are expressed within the cochlea at three locations: the OC, the spiral ganglion (SG) and the stria vascularis (SV). MMP-2 had an equally distributed gene expression in the cochlea while MMP-9 mRNA expression was particularly highly in the SG. We also observed by immunofluorescence their specific location mainly in inner and outer hair cells (HCs) and in the SG. OCs treated with 0.25 mM gentamicin showed an up-regulation of MMP-2 and MMP-9 protein levels after 24 hours of exposure with no change in their relative mRNA expression after 12, 24 and 36 hours of gentamicin exposure. Inhibition of the MMP activity in OCs incubated with an MMP inhibitor in organotypic OC cultures resulted in HC death. Conclusions: We demonstrate that both active and proactive forms of MMP-2 and MMP-9 are expressed in the cochlea. Using immunohistochemistry, we were able to locate these two MMPs specifically within the OC and the SG of the inner ear. Both MMP-2 and MMP-9 active forms were up regulated after aminoglycoside exposition, which suggest mainly a posttranscriptional regulation after gentamicin treatment since we did not observe a change in the corresponding mRNA levels. HC death after MMP inhibition in vitro remarks that a basal level of MMP activity is required for HC survival.

Ototoxicity and bacteriostatic activity of Burow's solution

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PURPOSE: Burow's solution (13% aluminium acetate) has been increasingly recommended as a topical otologic solution for the treatment of intractable chronic suppurative otitis media, including methicillin resistant S. aureus, P. aeruginosa and fungal infection. The ototoxicity of this solution, however, has not been reported. The purpose of this study is to evaluate the toxic and bacteriostatic effects of this solution. Because Burow's solution contains acetic acid, the ototoxicity of acetic acid was also studied.

MATERIAL AND METHOD: Ototoxicity was evaluated in guinea pigs using the eighth nerve compound action potential (CAP) with an electrode on the round window. The stimulus consisted of click sounds and tone bursts of 4 and 8 kHz. The middle ear of the animals were filled with Burow's solution, varying from full strength to several dilutions, and the reduction in the CAP was measured at 30 minutes, 1 hour, 2 hours and 24 hours. In another group of guinea pigs, the middle ear of the animals were filled with acetic acid at pH 4 and pH 5, and the reduction in the CAP was measured at 24 hours and one week. The bacteriostatic effect was studied by using MRSA, P. aeruginosa, S. pneumoniae, M. catarrharis, and H. influenzae. They were cultured on an agar plate and the diameter of the bacteriostatic ring on the agar plate was measured.

RESULTS: Burow's solution was found to be ototoxic at full strength. Burow's solution prepared at half strength did not show any ototoxic effects. The bacteriostatic activity of Burow's solutions was distinctly concentration dependent. Acetic acid at pH 4 caused a complete abolishment of CAP by 24 hours. Acetic acid at pH 5 by 24 hours caused a partial reduction in CAP for the tone bursts of 4kHz, with no reduction for the tone bursts of 8kHz or the click stimulus. Acetic acid at pH 5 by one week caused a partial reduction in CAP for the tone bursts at 4 kHz and 8 kHz with no reduction for the click stimulus.

CONCLUSION: Ototoxicity, as well as bacteriostatic activity of Burow's solution was concentration dependent and time dependent. Burow's solution at half strength did not show any ototoxic effects, yet retained adequate bacteriostatic properties, hence we believe this solution when diluted to half strength can be safely used in the clinical setting. The thinner round window membrane found in guinea pigs are likely to be a factor that could increase the observed ototoxic effects of full strength Burow's solution than would be expected to be seen in humans. However, these results warrant a caution in the use of Burow's solution in humans particularly in the pediatric population.

Evaluation of hyperbranched Poly-L-Lysine uptake and toxicity on inner ear derived cell line as a function of concentration and architecture

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Several studies have examined the possibility to use non viral drug carriers in the inner ear. In the present study we introducedd an innovative and high potential system for plasmid delivery to inner ear, based on hyperbranched poly-L-lysine. We firstly evaluated the toxic side effects of polycationic macromolecules with different structure and molecular weight, and their uptake, to understand if they could be considered a good delivery system for potential inner ear application. OCk-3, from the inner ear of ImmorthomouseTM were treated with three different hyperbranched Poly-L-Lysine (HBPL), and with Polyethylenimine (PEI) in a dose range between 10-11 and 10-5 M, for 3, 6 and 24 h. The uptake and cell viability were measured by fluorescent microscopy and flow cytometry. Polycations were taken up by cells in a time and dose-dependent way. As expected, cell viability decreased with increasing sample concentrations. Apoptosis mechanisms underlying cytotoxicity were investigated by immunocytochemistry, evaluating the release of Cytchrome c from mitochondria. The results supported the hypothesis that cell death occurred by apoptosis, in a dose and time dependent way, although nuclear fragmentation was not detected. Moreover HBPL toxicity increased with increasing molecular weights, but it was less toxic than comparable PEI. After verifying the HBPL ability to complex DNA, using a Math1-GFP plasmid, we evaluated the mechanism trough which these complexes are taken up by the cells and their expression. These results suggested us that at low concentrations HBPL could be useful as a non-viral system for drug delivery in the inner ear.

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Manufacturing and *in vivo* inner ear visualization of MRI traceable multifunctional liposome nanoparticles tagged by gadolinium

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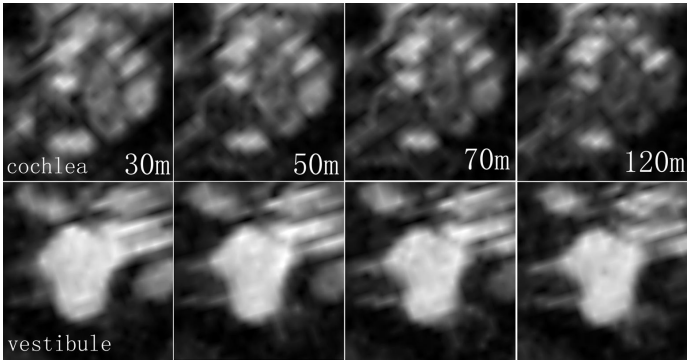
Treatment of inner ear diseases remains a problem because of limited passage through the blood-inner ear barriers and uncontrollable delivery of treatment agents after either intravenous or oral administration. As a minimally-invasive approach, intratympanic delivery of multifunctional nanoparticles (MFNPs) for the administration of genes or drugs to the inner ear is a future treatment option for diseases of the inner ear, including sensorineural hearing loss (SNHL) and Meniere's disease. In an attempt to track the dynamics and distribution of MFNPs in vivo, MRI traceable liposome nanoparticles were developed by encapsulating gadolinium-tetra-azacyclo-dodecane-tetra-acetic acid (Gd-DOTA) (abbreviated as LPSGds). Measurements of r1 and r2 relaxivities showed that LPSGds had efficient visible signal characteristics for MRI. In vivo studies demonstrated that LPSGds with 150 nm size were taken up by the inner ear after transtympanic injection. With intracochlear injection, LPSGds were visualized to distribute throughout the inner ear, including the cochlea and vestibule with fast dynamics depending on the status of the perilymph circulation. In conclusion, novel LPSGds were visible by MRI in the inner ear in vivo demonstrating transport from the middle ear to the inner ear and with dynamics that correlated to the status of the perilymph circulation.



The passage of nanoparticles from the middle ear to the cochlea in the rat

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The inner ear is an isolated, multicompartmental organ. To apply drugs directly to the cochlea runs a risk of causing irreversible damage. The less invasive approach is to apply a drug to the middle ear and transport it through the round window membrane (RWM) to the cochlea.

Six types of nanoparticles (NPs) were tested: liposomes (University of Helsinki, Finland), polymersomes (University of Southampton, UK), silica NPs (Abo Akademi University, Turku, Finland), hyperbranched polylysines (EPFL, Lausanne, Switzerland), poly(lactide-co-glycolide) PLGA NPs (KTH, Kista, Sweden) and lipid core nanocapsules (University of Anger, Anger, France). NP size ranged between 30 – 100 nm. NPs were tagged with a fluorescent dye (rhodamine, DiO, fluorescein, or CdSe/CdS quantum dots). NPs were applied on the small piece of gelfoam on the RWM in ketamine (35 mg/kg) and xylazine (6 mg/kg) anaesthetized rats or mice either as a single injection of a 5 – 10 µl suspension or using continuous delivery with an Alzet microosmotic pump (100 – 200 µl of NP suspension for 7 – 14 days). The animalss' hearing thresholds were periodically assessed with auditory brainstem responses (ABR), while the physiological state of the outer hair cells was assessed by recording distortion-product otoacoustic emissions (DPOAEs). Histological evaluation was performed at the end of the experiment. Animals were sacrificed, their auditory bullas were fixed in paraformaldehyde (4%) and decalcified. Paraffin-embedded sections of the cochlea (10µm) were stained with Alexa Fluor 488-labeled phalloidin and DAPI. A confocal microscope (Zeiss 510 DUO) was used for analysis of the samples.

All types of NPs were identified within 24 hours in the cochlea. They entered the cytoplasm of cells in the organ of Corti (hair cells and supporting cells), neurons of the spiral ganglion and cells of the lateral wall. NPs did not cause any distinct morphological damage in the inner ear nor any pronounced changes in hearing threshold or DPOAE amplitudes. For this reason nanoparticles seem be suitable drug transporting tool to the inner ear.

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Cause of sudden hearing loss (Where's the energy?)

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Cochlear sounds are longitudinal vibrational waves in the perilymph that transport energy. The cochlea is a closed system and sound energy that enters at the stapes-oval widow, is transduced and stimulates the receptor hair cells, travels through the compliant basilar membrane (BM) and is released at the round window (RW). Any additional opening in the cochlea wall allows a diversion of sound energy and results in a sudden or fluctuating hearing loss, fullness in the ear and/or tinnitus. Perilymphatic fistulas caused by trauma may allow the leakage of perilymph into the surrounding area and produce a hearing loss. Experimental openings in the bony wall to study neural or BM activity result in a raised threshold. The onset of an idiopathic, sudden sensorineural hearing loss (SSHL) is characterized by a loss that develops within 72 hours and it may occur without an apparent fistula or loss of perilymph. An infection or injury that affects the bony wall (also think osteoporosis) or the BM may cause an area to be thinner or weaker so that sound energy can escape (i.e. be vented). Such a SSHL may be difficult to image or identify because there is no apparent injury to hair cells, BM or other structure and no loss of perilymph. Treatments should alleviate the cause (eg. infection) and repair the tissue by stimulating regrowth (eg. osteoblasts). An associated loss of hearing often occurs after an injured RW has been repaired to preventing perilymph loss. The RW is the normal release structure for proper passage of sound energy through the closed cochlea. Just as there should be no extra vents, there must be one vent with the proper compliance to allow sound waves to pass through and stimulate the receptor hair cells. Care is needed to seal the RW with a material of the proper compliance – if possible, using remaining RW tissue is preferable.

A CCTTT-repeat at the promoter of NOS2A gene confers protection to Ménière`s disease in two cohorts of european population

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HYPOTHESIS: Hearing loss in Ménière disease (MD) is related with loss of spiral ganglion neurons (SGNs) and hair cells. In a guinea pig model of endolymphatic hydrops, nitric oxide synthases and oxidative stress mediate loss of SGNs. Since the length of a CCTTT microsatellite at the promoter of NOS2A gene determines its transcription rate, we studied the association of this repeat with MD.

STUDY DESIGN: A case control study.

METHODS: We genotyped a CCTTT repeat (rs3833912) at the promoter of NOS2A gene in two independent cohorts including a total of 268 patients with MD and 510 controls.

RESULTS: Shorter alleles CCTTT with 6-8 repeats were significantly more frequent in mediterranean (OR=0.36 (CI, 0.20-0.69), corrected p=0.022) and Galicia (OR=0.08 (0.02-0.27), corrected $p=1.8x10^{-7}$) controls than in patients with MD. Metanalysis estimated that the (CCTTT)6-8 repeats occurred in 11% of controls and 3% of patients (0R=0.23 (0.13-0.40), corrected $p=1.1x10^{-8}$). Cochran-Armitage trend test found an association between the number of CCTTT repeats and the risk for MD ($p<10^{-4}$).

CONCLUSIONS: Our data show that the shorter CCTTT repeats at NOS2A promoter may protect against MD.

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Two Portuguese cochlear implanted dizygotic twins: a case report

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This study aimed at performing clinical and genetic characterization, as well as evaluating the oral (re)habilitation, of two dizygotic twins with cochlear implant (CI). The twins presented neurosensorial profound deafness prior CI (age of implantation = 3.5 years old). Both parents are mute-deaf and have one unaffected daughter each from a prior marriage, as well as several affected and unaffected relatives. The two twins and their parents were analysed in respect to the presence of GJB2/GJB6 mutations. PCR-site directed mutagenesis was first performed to detect the common 35delG mutation in the GJB2 gene. When necessary, direct sequencing of the entire coding region was further performed. Multiplex PCR was used to detect the GJB6 deletions del(GJB6-D13S1854) and del(GJB6-D13S1830). Assessment of the twins' global oral performance after cochlear implantation was carried out by applying a battery of speech and audiological tests. The twin girl and the father were 35delG homozygotes while the twin boy and the mother were 35delG/del(GJB6-D13S1830) compound heterozygotes. The genetic cause of deafness was thus identified in the four members of this family. As regards the twins' oral performance after CI, some noteworthy differences were observed. While the twin girl had an overall good performance throughout the tests, the twin boy got poorer results and wasn't even able to reply to some of the audiological tests performed. The dizygotic twins here analysed, aged 8 years, presented different genotypes (35delG/35delG and 35delG/ del(GJB6-D13S1830)) and different oral performance after CI. To our knowledge, this was the first time that del(GJB6-D13S1830) deletion was found in Portuguese deaf patients. The observed differences in the oral performance are most probably due to the different social context in which the twins have been living and not to their different GJB2/GJB6 genotype: the twin girl has been living with a hearing aunt, while the twin boy lived until very recently with the parents, in a poor auditory stimulating environment with other problems, such as parents' alcoholism. The remaining twins' affected relatives will probably carry either the 35delG mutation and/or the del(GJB6-D13S1830) deletion. Molecular diagnosis and genetic counselling is very important to a family such as this one, namely to the twins' unaffected half-sisters, who are certainly carriers of one of these mutations.

The GJB2 gene and hearing loss: beyond the coding region

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INTRODUCTION: In many populations, a significant percentage of non-syndromic, sensorineural, prelingual hearing loss cases are due to mutations in GJB2/GJB6 genes (DFNB1 locus). Thus, molecular diagnosis of hearing loss (HL) is usually initiated by searching mutations in GJB2 coding region and flanking sequences, including the acceptor splice site. The donor splice site is often analysed, mainly aiming at the identification of the pathogenic mutation c.-23+1G>A which has been reported in several hearing loss cases. If necessary, screening for GJB6 deletions del(GJB6-D13S1830) and del(GJB6-D13S1854) is further performed. Analysis of exon 1 of GJB2 has been performed in some studies while the whole 3'UTR of the gene has been analysed only in a very small number of patients. No pathogenic mutation has ever been reported in these two GJB2 non-coding regions. Recently, a second pathogenic non-coding GJB2 mutation (c.-259C>T) has been identified in the basal promoter, highlighting the relevance of searching for non-coding mutations in hearing loss cases. A 10 bp deletion (c.-684_-675del, firstly designated -493del10), occurring upstream of the GJB2 basal promoter, with so far unknown pathogenicity, has also been reported, and is in linkage disequilibrium (LD) with the c.101T>C mutation in the coding region of the gene.

METHODS: We have sequenced GJB2 promoter, exon 1 and 3'UTR from 89 Portuguese HL patients, previously screened for GJB2 coding region and GJB6 deletions, and 91 hearing individuals from the control population.

RESULTS: We have genotyped all individuals for: the c.-684_-675del deletion; three SNPs in the promoter (rs9550621, rs73431557, rs9552101); 10 SNPs in the 3'UTR (c.*1C>T, rs3751385, rs7337074, rs7329857, rs55704559, rs5030700, rs1050960, rs7623, rs11841182 and rs7988691); and one SNP downstream of the 3'UTR end (rs11839674). We have identified in the hearing control sample a c.-684_-675del homozygote (not harbouring c.101T>C), supporting the non-pathogenicity of this deletion in homozygosity. Moreover, we have observed a statistically significant difference between patients and controls, in allelic frequencies relative to four of the 14 SNPs genotyped: rs73431557, rs3751385, rs55704559and rs5030700. Regarding rs55704559 and rs5030700 (at c.*168 and c.*931 positions respectively), nearly all individuals analysed (178/180) are either c.[=;=]+[=;=] or c.[*168A>G(+)*931C>T]. This results from LD between rs55704559 and rs5030700 SNPs (Chi2=160.8480, pG(+)*931C>T] individuals is significantly higher in patients than in controls (p=3.36293 E-06). The statistically significant differences in rs73431557 and rs3751385 allelic frequencies are mostly due to LD between these SNPs and the rs55704559 and rs5030700 SNPs. Interestingly, the change c.*168A>G, but not c.*931C>T, is predicted to alter mRNA folding (regardless of the genotype at the other position). Therefore it is possible that variant c.168A>G contributes to HL. Alternatively, allele c.[*168G;*931T] may be in LD with an unknown pathogenic variant.

Cerebrocortical plasticity after acute and chronic unilateral lesion of the central vestibular system in rats: a micro-PET study

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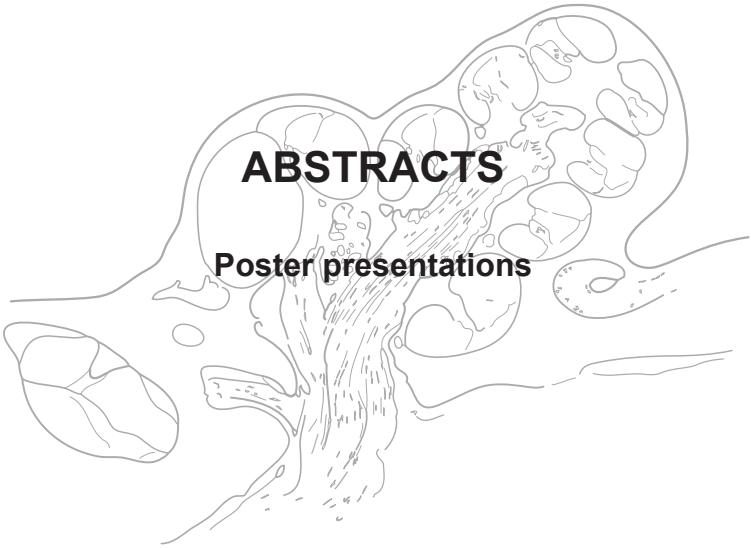
OBJECTIVE: The purpose of this study was to investigate glucose metabolism of vestibular processing cerebrocortical and subcortical brain regions in rats after acute and chronic unilateral lesion in the central vestibular pathway.

METHOD: An electrolytic lesion was applied to a vestibular processing thalamic nucleus and was verified via MRI scan immediately after operation. The glucose metabolism during vestibular stimulation was investigated using 18F-Fluorodesoxyglucose (FDG) tracer. Brain activity of eight lesioned and four control rats was measured under six conditions in Micro-PET Focus 120: 1. Control stimulation; 2. left galvanic vestibular stimulation; 3. one day, 4. three days, 5. seven days and 6. twenty days post lesion upon left vestibular stimulation. Vestibular stimulation was performed by electrical stimuli applied via electrodes (anodal: external auditory meatal cartilage; cathode: parietal subperiost), (t=50', 0.2 mA, 1 Hz). PET imaging started 60 min after i.p. injection of 25-32 MBq FDG for 30 min. By using the image pre-processing routines of Statistical Parametric Mapping (SPM), the images were realigned to a MRI image of the rat brain, and a FDG template was generated. After spatial normalisation to FDG template, voxelwise analyses (paired t-tests) with SPM were conducted.

RESULTS: The FDG-PET showed increased glucose metabolism in distinct brain regions when the activity upon stimulation in lesioned animals were compared to the pre-lesioned state. In particular, we observed a bilateral activation of the cerebellum that was seen at day 1 and persisted to day 20. We also found that the contralateral superior colliculus was activated from days 3-7 but not at day 20.

CONCLUSION: Our results suggest that parts of the cerebellum and visual system may respond with augmented activity when the vestibular pathway is affected, and reveal that compensating mechanisms may exist within the network that controls vestibular function.

Cornelis CORT (1536 – 1578)
Frans FLORIS (1517 – 1570)
Hearing
(National Gallery, Prague)



ABSTRACTS

Poster presentations

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Microarray analysis of differential gene expression along the tonotopic axis of mouse cochlea

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The cochlear map is characterized by tonotopic organization along its longitudinal axis, such that the base of the cochlear duct is more sensitive to high frequency sounds and the apical cochlea to low frequency sounds. As a first step to elucidate the mechanism by which the tonotopic organization is established, we identified the genes that are differentially expressed along the cochlear duct using microarray analysis. Cochlear tissues containing the organ of Corti were dissected from some fifty P8 C57BL/6 mice, and divided into three pieces representing the base, middle and apex of the cochlear duct. Total RNAs were isolated from each pool and the gene expression profiles were analyzed using the Affymetrix GeneChip® Mouse Gene 1.0 ST Array. We identified 55 genes expressed in an increasing gradient from the base to the apex with at least two fold difference and 52 genes expressed in a decreasing gradient. Among the genes expressed more abundantly in the apical cochlea are proteins involved in stereocilia formation such as Usher syndrome 1C homolog (ush1c), protein tyrosine phosphatase receptor type Q (ptprq), and myosin binding protein C (mybpc1). Genes involved in synaptic transmission (e.g. synaptotagmin, syt1; nicotinic cholinergic receptor, chrna10) and ion transport (e.g. stanniocalcin 1, stc1; K^+ channel, kcnh8; solute carrier organic anion transporter, sloc5a1) were also expressed highly in the apical cochlea. Conversely, the genes expressed more abundantly in the base of the cochlear duct include proteins associated with ion transport (e.g. transmembrane protein, tmem196; pannexin3, panx3; solute carrier family member, slc26a7) and with electron transport (e.g. metaxin2, mtx2; NADH dehydrogenase (ubiquinone)1, ndufc1). We are currently confirming the microarray results with RT-PCR and also examining expression patterns of some of the genes of interest in the developing cochlea with in situ hybridization. Our data may provide an insight into the underlying mechanism of the tonotopic organization of the mammalian cochlea duct.

TMC1-Associated deafness in the Portuguese population

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INTRODUCTION: The TMC1 gene is located in the DFNB7/DFNB11 locus on 9q21.12 and encodes the transmembrane channel-like 1 protein of unknown function. TMC1 is expressed in human foetal cochlea. Mutations in this gene were shown to cause autosomal recessive non-syndromic hearing impairment (ARNSHI), as well as autosomal dominant HI.

OBJECTIVES: The aim of the present study was to determine the contribution of TMC1 mutations to ARNSHI in the Portuguese population.

METHODS: In order to identify TMC1 mutations, 15 of the 20 coding exons and flanking intronic sequences of the TMC1 gene were analyzed by dHPLC for 185 unrelated probands which deafness is not associated to DFNB1. Samples with patterns consistent with heterozygosity were further sequenced for the corresponding exon and for exons not analyzed by dHPLC.

RESULTS AND DISCUSSION: Preliminary results indicated high frequency of mutations (3 to 7%) in TMC1 in the Portuguese probands with ARNSHI. However, further analysis revealed that most exonic and intronic nucleotide changes are in fact polymorphisms. As such, TMC1 mutations are not a major cause of ARNSHI in our population.

This was the first screening of DFNB7/11-related deafness in the Portuguese population.

Screening of mitochondrial DNA mutations involved in hearing impairment

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Mutations in mitochondrial DNA (mtDNA) are known to be associated with sensorineural hearing loss. Inherited and sporadic mtDNA mutations have been identified in both syndromic and nonsyndromic hearing loss as well as in predisposition to aminoglycoside-induced ototoxicity. We report a systematic mutational screening of 4 specific mitochondrial DNA regions in 185 probands including hearing impaired patients and some of their familials. In particular we took into consideration the genes most frequently involved in hearing loss such as 12S rRNA, 16S rRNA, tRNA valine, tRNA leucine, NADH dehydrogenase subunit I ,cytochrome c oxidase subunit I, tRNA serine, tRNA aspartic acid, cytochrome c oxidase subunit II. Blood and saliva samples were collected and the sequences pcr-amplified and analyzed in comparison to the Cambridge reference sequence. Mutations known to be causing deafness were found and some polymorphisms and de novo mutations were detected. Some patients showed the presence of more than one mutation in their mtDNA and in most of them the maternal inheritance was confirmed. Of the 90 mtDNA variants we could identify, 70 are known and 20 novel. Six caused missense mutations not found in literature. The frequencies of known A1555G and T961G mutations were 1.62% and 3.24% respectively, in this cohort with nonsyndromic and aminoglycoside-induced hearing loss.

Connexin 26 and connexin 30 immunostaining in wild type and Cx30T5M knock-in mice

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Non–sensory cells in the cochlea form intercellular networks coupled by gap junction (GJ) channels composed primarily of connexin 26 (Cx26) and connexin 30 (Cx30) protein subunits (1), which appear to be coordinately regulated (2). Connexins also form hemichannels which release ATP that subtends propagation of intercellular calcium signals (3). In cochlear preparations from P5-P6 wild-type mice, our immunolabelling of Cx30 and Cx26 showed a gradient in outer sulcus (OS) cells (i.e. non-sensory cells lateral to the inner hair cells) markedly decreasing along the longitudinal axis, from the basal to the apical end of the cochlea. The gradient was less evident in inner sulcus cells (i.e. non-sensory cells medial to the inner hair cells). Immunofluorescence signals corresponding to connexin hemichannels were identified using antibodies against Cx26 extracellular loop peptides (CELAbs) (4) and localized to the apical plasma membrane of cochlear non-sensory cells. In cochleae from P7 mice, connexins were detected mostly in the apical surface of tall columnar cells in the Kölliker organ, which disappears toward the onset of hearing (5), and were only sparsely expressed in the core of the organ. In contrast to Cx30(-/-) mice, in which Cx30 expression is ubiquitously abrogated (6) and Cx26 is downregulated (2), in adult (P30) Cx30T5M/T5M mice the mutated protein was still expressed and appeared to be correctly targeted, compared to age matched wild-type mice. Likewise, Cx26 immunostatining appeared unaffected in Cx30T5M/T5M mice.

1. Nickel, R., and Forge, A. (2008) Curr Opin Otolaryngol Head Neck Surg 16, 452-457

2. Ortolano, S., Di Pasquale, G., Crispino, G., Anselmi, F., Mammano, F., and Chiorini, J. A. (2008) Proc Natl Acad Sci U S A 105, 18776-18781

3. Anselmi, F., Hernandez, V. H., Crispino, G., Seydel, A., Ortolano, S., Roper, S. D., Kessaris, N., Richardson, W., Rickheit, G., Filippov, M. A., Monyer, H., and Mammano, F. (2008) Proc Natl Acad Sci U S A 105, 18770-18775

4. Clair, C., Combettes, L., Pierre, F., Sansonetti, P., and Tran Van Nhieu, G. (2008) Exp Cell Res 314, 1250-1265

5. Hinojosa, R. (1977) Acta Otolaryngol 84, 238-251 6. Teubner, B., Michel, V., Pesch, J., Lautermann, J., Cohen-Salmon, M., Sohl, G., Jahnke, K., Winterhager, E., Herberhold, C., Hardelin, J. P., Petit, C., and Willecke, K. (2003) Hum Mol Genet 12, 13-21

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Generation of a tamoxifen inducible hair cell specific TR(beta)1 knock-out mouse model

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Thyroid hormone receptor (beta)1 (TR(beta)1) dysfunction leads to deafness in humans and mice (Refetoff et al. 1993; Forrest et al. 1996). Deafness in TR(beta)1 mutant mice has been suggested to result from TR(beta)1-mediated control of fast-activating BK currents in inner hair cells (Rüsch et al. 1998). New results, however, suggest that deafness is not a result of delayed BK currents, but may have an origin outside the hair cells (Winter et al. 2009). To further verify this presumption, we aimed to delete the TR(beta)1 receptor restrictively in hair cells, within a critical time period prior to the onset of hearing. To obtain a hair cell-specific deletion of TR(beta)1, we use a well-established CreLoxP system and a transgenic mouse model (Math1CreERTM), in which the expression of the Cre-recombinase is under control of the Math1 promotor and its activation is inducible by Tamoxifen (Chow et al., 2006). In the cochlea, Math1 is only expressed in hair cells, from E13 to P7, therefore Cre expression and gene deletion is presumed to be active during that time. This makes it possible to generate mice with a hair cell specific deletion of TR(beta)1 prior to the onset of hearing. By crossing Math1CreERTM mice with floxed TR(beta)1 mice, the obtained mouse model is ready for functional and cellular phenotyping. First data will be presented that describe the hearing function of these inducible hair cell specific TR(beta)1-knock-out animals as well as the phenotype of hair cells.

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Intracochlear injection of adeno-associate virus vector to a mouse model created by a conditional knockout of Gjb2 gene

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Hereditary deafness affects about 1 in 2,000 children and mutations in the gap junction beta 2 protein (GJB2) gene are the major cause in various ethnic groups. In order to establish the fundamental therapy of congenital deafness, we generated targeted disruption of Gjb2 using Cre recombinase controlled by P0. Using this animal model, we examined the potential of gene therapy in the inner ear, using the homozygous mutant mice and the heterozygous mutant mice. Adeno-associated virus vectors carrying the Gjb2 gene were injected into the scala tympani through the round window of the cochlea of the homozygous mutant mice. The Cx26 was observed in the fibrocytes of the spiral ligament and spiral limbus, but the expression of Cx26 was not seen in the supporting cells and did not improve the hearing ability. We succeed in gene introduction to the supporting cells of neonatal mice without hearing loss using adeno-associated virus vectors (2008, Iizuka T, et al.). We are going to introduce this virus into the Gjb2 knockout mouse in future to cure hereditary deafness.

Cochlear gap-junction plaque is disrupted by dominant-negative connexin26 mutation

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Hereditary deafness affects about 1 in 2000 children and mutations in the GJB2 gene are the major cause in various ethnic groups. GJB2 encodes connexin26 (Cx26), a channel component in cochlear gap junction. It has been hypothesized that gap junction in the cochlea, especially connexin26, provide an intercellular passage by which K^+ are transported to maintain high levels of the endocochlear potential essential for sensory hair cell excitation. We have reported the generation and the phenotype of a mouse model carrying human connexin26 with R75W mutation (R75W Tg mice). In this study, we analyzed the formation of gap junction in cochlear supporting cells of R75W Tg mice in different stages by confocal microscope. Gap junction composed of Cx26 in wild type mice showed horizontal linear gap-junction plaques along the cell-cell junction site with the adjacent cells and these formed pentagonal or hexagonal outlines of normal inner sulcus cells and border cells. The gap-junction plaques in R75W Tg mice did not show normal linear structure, but the round small spots were observed around the cell-cell junction site. A number of these small spots were scattered around their cell-cell junction site. We also observed the formation of the gap junction plaques in the primary culture of the organ of corti including inner sulcus cell and border cell. In both primary culture and co-culture of these cells, gap junction plaques were properly formed at the cell junction site. In vitro expression system for WT and mutant R75W also showed normal organization of their gap junction plaques. Regarding the disruption of gap junction plaques in mutant cochlear tissue, the assembly of the connexons may decline due to R75W mutation in Cx26 and it may cause mis-localiazation of the connexons. Our results may suggest that dominant negative R75W mutation affects on the accumulation and localization of gap junction channels in the cell-cell junction site among cochlear supporting cells.

Role of neural crest and Pax3 in mammalian inner ear development

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The vertebrate inner ear develops from a specialized region of the ectoderm located on either side of the caudal hindbrain known as the otic placode. During development, the otic placode invaginates to form the otic cup, from which some cells delaminate and migrate into neighboring mesenchyme to form neurons of the cochlear-vestibular ganglion. The otic cup deepens further and pinches off from the ectoderm to form the otocyst, which over time develops into the membranous labyrinth of the inner ear. While a majority of the cells in the membranous labyrinth are derived from the otic placode, some of the cells in the labyrinth are derived from the neural crest. Neural crest originated from the junction between the epidermis and dorsal region of the neural tube gives rise to a variety of cell types in the embryo such as neurons, glia, melanocytes, bones and cartilages. To identify the neural crest contribution to the mouse inner ear, we genetically fate mapped progeny of neural crest in the inner ear using Pax3-Cre mice. Pax3 is a member of the Pax family of transcription factors, and it is important for various aspects of embryogenesis including neural crest differentiation. In human, mutations in PAX3 cause Waardenburg's syndrome Type I, characterized by neurosensory hearing loss. Pax3 is expressed in the dorsal neural tube, which includes the neural crest, but it is not expressed in the developing inner ear. Crossing Pax3-Cre with R26R reporter mice, we identified descendants of Pax3-expressing cells in the inner ear of Pax3-Cre; R26R compound heterozygotes using β-gal histochemistry. At E17.5, β-gal positive cells are detected in various regions of the inner ear including endolymphatic duct, stria vascularis, and ganglia. In addition, β-gal positive cells are present in some parts of the otic capsule and in all three middle ear ossicles. Furthermore, analyses of Pax3-Cre homozygous mutant embryos showed that Pax3 is required for melanocytes formation in the stria vascularis but not other neural crest-derived cell types in the inner ear.

Role of RAF kinases in inner ear development

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Insulin-like growth factor (IGF) I is fundamental for the regulation of cochlear growth and differentiation, and its mutations are associated with hearing loss in mice and men (1, 2). IGF-I actions are mediated by intracellular signalling networks one of which is the RAF/MAPK pathway. RAF kinases are involved in a wide variety of cellular processes such as differentiation, proliferation and apoptosis. RAF kinases have redundant functions in cellular homeostasis, but interestingly they also have cellular and tissue-specific functions (3). Indeed, C-RAF activation is a critical step in the modulation of the proliferative stages of the chicken otocyst (4). These data suggest a key role of RAF kinases in the developing inner ear. To explore this, we have studied the expression profiles of RAF kinase transcripts and proteins during inner ear development in mice and chicken. Our data indicate a strict control of RAF kinase levels during development. We have also studied whether the deficit of these kinases activity has an impact on inner ear development and/or function in both species. In this work we show that the inhibition of RAF activity in ex vivo cultures of chicken otic vesicles increases apoptosis, abolishes proliferation, significantly reduces early otic neurogenesis and impairs axon elongation. On the other hand, ABR studies indicated that hearing is severely impaired in young cRaf-1 knock out mice, which presented an auditory threshold of 90 dB SPL. This neurosensorial deafness is concomitant with the reduction of the potassium channel Kir 4.1 in the stria vascularis. In summary, these results demonstrate that RAF kinases play a pivotal role in inner ear development and function. This work was supported in part by the Instituto de Salud Carlos II, Centro de Investigación en Red en Enfermedades Raras CIBERER, and MICINN (SAF2008-00470).

(1) Sanchez-Calderon et al. Int J Dev Biol, 5:557. 2007. (2) Cediel et al. Eur J Neurosci, 23:587. 2006. (3) Camarero et al. Mol Cell. Bio, 26(19), 7103-7115.2006. (4) Sanz et al. Oncogene, 18(2), 429-437. 1999.

Differential requirement of Pou3f4 in the development of cochlear lateral wall: A study on a mouse model for DFN3

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DFN3 is an X chromosome-linked nonsyndromic deafness and characterized by a conductive hearing loss, a flow of perilymph during the opening of the stapes footplate, and progressive sensorineural deafness (Nance et al., 1971; Cremers et al., 1985). DFN3 is known to be associated with mutations of POU3F4, which encodes a member of the POU family transcription factors (de Kok et al., 1995). Pou3f4, the mouse orthologue of human POU3F4 gene is expressed in the mesenchyme surrounding the inner ear (Phippard et al., 1998). Consistently, previous studies using Pou3f4 knockout mice have shown anomalies in the mesenchymal-derived tissues such as temporal bone and otic fibrocytes of the cochlear lateral wall. To further understand pathological mechanisms of hearing loss in DFN3, we analyzed the inner ears of Pou3f4del-J mice (The Jackson Laboratory), which carries a spontaneous deletion in the Pou3f4 locus. We have examined developmental series of the inner ear defects of the mutants, focusing on the otic fibrocytes and stria vascularis in the cochlear lateral wall, both of which are essential for sensorineural component of hearing. Our observations suggest that the onset and pattern of the defects of the cochlear lateral wall are different between the otic fibrocytes and stria vascularis.

Expression analysis of prestin and selected transcription factors in newborn rats

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The high sensitivity of hearing depends strongly on the function of special mechanosensory cells – the outer hair cells (OHCs) - which amplify sound-induced vibrations in the organ of Corti by the motor protein prestin. The molecular mechanisms that regulate the expression of the prestin gene are poorly understood. Transcription factors have a central role to play in regulating gene expression. In this study, we compared the expression pattern of the transcription factors Brn-3c (member of the POU family of transcription factors), Cebp-beta (CCAAT/enhancer binding protein beta, member of the basic-leucine zipper class of DNA binding proteins), Sp1 (Specificity protein 1, family of zink finger DNA binding transcription factors), Carf (calcium response factor) and Creb (cAMP response element binding protein) with that of prestin. We selected and grouped these transcription factors according to the grouping suggested by Brivanlou and Darnell [1]: (i) Brn-3c as a transcription factor specifically associated with the developmental regulation of the inner ear, (ii) Cebp and Sp1 as constitutive acting transcription factors which function as enhancers or repressors and (iii) Carf and Creb as signal dependent transcription factors. To analyze the role of these transcription factors for the transcriptional regulatory mechanism of prestin, we applied an RT-PCR approach combining three kinds of information: (i) expression changes during postnatal development, (ii) expression changes along the apical-basal gradient and (iii) expression changes by exposure of organotypic cultures of the organ of Corti to factors which significantly affect prestin expression. We found that the Brn-3c mRNA levels are significantly associated with the up- and down-regulation of prestin under most of the analyzed conditions. Brn-3c and Cebp are the only transcription factors involved in the formation of the apical-basal gradient. In thyroid hormone (T4) supplemented cultures, we observed a parallel increase of prestin, Brn-3c and Cebp expression. In KCl treated cultures, the decrease of prestin expression was associated with the decrease in Carf and an increase of Creb mRNA levels. Butyric acid which changes the histone acetylation status of the chromatin increases both prestin and Brn-3c expression in parallel. The present results together with our previous findings indicate that prestin expression in newborn rats is affected on the transcriptional and posttranscriptional levels. The transcription factors Brn-3c and Gata-3 are candidates for regulating prestin at both levels.

[1] A.H.Brivanlou, J.E.Darnell, Jr., Signal transduction and the control of gene expression, Science. 295 (2002) 813-818.

Immunological and genetical influence of systemic helper T cell on age-related cochlear functions

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Functional decline of the nervous and immune systems are cardinal features of aging. In the cochlea, age-related loss of hair cells and spiral ganglion neurons is a major contribution to age-related hearing loss (presbycusis). In the systemic immune system, age-related dysfunctions of helper T (Th: CD4-positive) cells come to the front of immune senescence as a result of the thymic involution. The senescence-accelerated mouse strain P1 (SAMP1) have been developed from the parental AKR/J mice, which shows an early occurrence of thymus atrophy and accelerated dysfunctions of immunocompetent cells, particularly Th cells, followed by accelerated hearing impairment with degeneration of spiral ganglion cells (SGCs), wrinkled skin, and increased lordokyphosis of the spine, as well as shortened lifespan. In the current study, we examined neuroimmune interaction between the cochlea and systemic cellular immune system using SAMP1. We inoculated Th cells into the syngeneic host or grafted the fetal thymus under the renal capsule of he host. These host mice showed delays of age-related development of hearing loss, degeneration of spiral ganglion (SG) cells, and immune deterioration in SAMP1. We also examined gene expression of Th cells of 2-months-old SAMP1, 8-months-old SAMP1, and 8-months-old SAMP1 which had been grafted fetal thymus under the renal capsle. Several Th- or CD4-related genes including an interleukin receptor gene were found to show upregulattion as aging, but supression by thymus graft. These findings indicate that management of systemic Th cell functions with immunological and/or genetical supports influence the cochlea and prevents presbycusis. Further studies into the relationship between age-related systemic immune functions and neuro-degeneration mechanisms may provide additional information pertinent to the treatment of age-related diseases.

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Degeneration in marginal cells of the ageing mouse stria vascularis

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Atrophy of the stria vascularis can contribute to hearing loss in human beings, and is the most consistent age-related change that has been found in post-mortem material. Age-related changes are also found in the stria vascularis of mice, and includes thinning of the stria, and in some strains, loss of marginal cells. In the present experiments, cochlear lateral walls including the stria vascularis of two strains of ageing mice were imaged by confocal microscopy, following staining with different markers. Actin was marked with fluorescently-conjugated phalloidin, mitochondria with Mitotracker Red and Mitotracker Green, and phosphorylated tyrosines with an antibody (Abcam ab50722). In ageing C57BL/6 mice, the stria vascularis became thinner, but there was no loss of marginal cells, as revealed by imaging the cell boundaries on the lumenal surface of the epithelium with the phalloidin actin marker. However, about 50% of the marginal cells showed markedly enhanced actin staining around their apical margins compared with their neighbours, apparently at random. In contrast, in BALB/c mice, not only did the strial epithelium become thinner, but there was a substantial reduction in density of marginal cells. Presumably to fill the gaps, the apical surface of some marginal cells expanded massively, while those of their neighbours were unchanged. The expanded cells tended to show lowered levels of actin staining in their apical margins, while the unaffected cells tended to show enhanced staining. Tyrosines become phosphorylated during cellular signal transduction. Staining for phosphorylated tyrosines showed sporadic enhancement in ageing marginal cells, particularly in the cells with the enhanced actin staining. However, this was not consistent – in some cases enhanced reaction was found in the expanded marginal cells, cells which also tended to show reduced actin staining. This contrasts with the inner ear mechanosensory epithelium where enhanced tyrosine phosphorylation is generally seen in the supporting cells surrounding a damaged hair cell, presumably preparatory to their repair response, which reseals the epithelium when the damaged cell is extruded. The present experiments may inform us of the mechanisms by which analogous repair and resealing are initiated in the stria vascularis.

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Activation of NF-κB-iNOS pathway in the lateral wall of aged cochlea

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Clinically, presbysusis is known to be progressive and irreversible. Production of free radicals and apoptosis is supposed to contribute the pathogenesis. Inducible Nitric Oxide (iNOS), which is one of free radicals, is reported to be controlled by Nuclear Factor kappa B (NF-κB). We investigated the activation of NF-κB-iNOS pathway in the lateral wall of aged cochlea using immunohistochemical and electrophysiological methods. 24 mice were used in this experiment. We recorded the hearing threshold by ABR at 1, 4, 8 and 12 months old. The tissues were fixed via cardiac perfusion with paraformaldehyde. The cochleas were incubated in the same fixative overnight. Decalcification was performed. Subsequently, the tissues were embedded in paraffin. They were incubated with the first antibody to iNOS or NF-κB. The elevation of hearing threshold was observed at 4-month-old. Then, the hearing threshold was worsen according to aging. NF-κB and iNOS were not detected at 1-month-old. NF-κB was expressed in the stria vascularis and spiral ligament at 4-month-old, however, iNOS was not expressed. NF-κB and iNOS were observed at 8-month-old and 12-month-old. We demonstrated that the hearing threshold shift was apparent at 4-month-old and the expression of NF-κB and iNOS at 8-month-old. NF-κB is a transcription factor which controls inflammatory genes including iNOS. The active forms of NF-κB are composed of two DNA binding subunits, p50 and Rel A. The activation of NF-κB involves two steps, that is, the release of an inhibitory IκB and the translocation of the activated form from the cytoplasm to the nucleus. We also showed that the administration of the anticancer drug, cisplatin, induced the expression of iNOS in the inner ear (Anticancer drugs, 2000). Stria vascularis and spiral ligament has important roles to maintain the homeostasis of inner ear. Our findings suggest that the damages on the stria vascularis and the spiral ligaments might be one reason for the functional disturbance caused by the aging.

Identification of Caprin-1 as a novel target of Pou4f3 and its role in cochlear hair cells in response to ototoxic damage

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The POU4 family of transcription factors are required for the survival of specific cell types in different sensory systems. Within the inner ear, Pou4F3 (also known as Brn-3c and Brn3.1) expression is restricted to the hair cells where it plays an essential role in inner ear development (Xiang et al, PNAS 1997); in Pou4f3-null mice hair cells fail to mature and undergo apoptosis resulting in mice without auditory or vestibular function (Xiang et al, Development 1998). Hair cell loss significantly contributes to the underlying pathology of many types of acquired hearing loss including ototoxicity, noise trauma and age-related hearing loss. Despite this, relatively little is known about the pathways regulating hair cell survival including those downstream of Pou4f3. Therefore to find novel targets of Pou4f3 we performed a subtractive hybridisation screen on an inner ear cell line (OC2 cells) with manipulated levels of Pou4f3 expression. This screen identified the cytoplasmic phosphoprotein Caprin-1 as a target of Pou4f3 regulation. Caprin-1 is highly expressed in several tissues including the brain where is localised to RNA granules in post synaptic dendrites and may play a role in regulating localised translation (Shiina et al, J Neurosci 2005). In addition, Caprin-1 is associated with cytoplasmic stress granules, another form of RNA granules that regulate translation in response to environmental stresses (Solomon et al, Mol Cell Biol 2007). Following the identification of Caprin-1 as a potential Pou4f3 target, we examined the Caprin-1 5‘ flanking sequence and using EMSA and ChIP analysis demonstrate that Pou4f3 can bind directly to regions within this sequence. Transient transfection experiments in OC-2 cells indicated that Pou4f3 represses Caprin-1 promoter activity, demonstrating for the first time direct Caprin-1 regulation by Pou4f3. As previously shown in other cell types, overexpression of wild type but not a mutated Caprin-1 in OC-2 cells was sufficient to induce stress granule formation. Further to this we examined Caprin-1 expression in the cochlea and found that Caprin-1-containing stress granules are induced in cochlear hair cells following damage by the aminoglycoside neomycin. This suggests that stress granule formation is an important hair cell damage response to a clinically relevant ototoxic agent. Determining Caprin-1 expression at the single cell level revealed that although expressed at low levels in native hair cells, Caprin-1 mRNA increases following aminoglycoside damage. Our data suggest a model in which a damage-induced reduction in, or modification of, hair cell Pou4f3 leads to the upregulation of Caprin-1 expression and stress granule formation in hair cells.

Evaluation of the potential meningitis risk of dexamethasone-eluting cochlear implants: A new animal model

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Preservation of usable residual hearing after cochlear implantation is essential for electric acoustic stimulation (EAS). In previous studies it was demonstrated that dexamethasone has a positive impact on hearing preservation. A drug-eluting silicone electrode-dummy loaded with 10% dexamethasone was developed in order to apply dexamethasone locally in an animal model. A rare but dangerous complication of cochlear implantation is meningitis following an acute Otitis media. The aim of this study was to compare dexamethasone-loaded silicone rods to unloaded silicone rods with respect to potential meningitis risk. In a first set of experiments a meningitis model for middle ear inoculation of Streptococcus pneumoniae D39 strain 2 (NCTC 7466) was established in the guinea pig after unilateral implantation of non-eluting silicone rods. Germ concentrations varying between 1x109 CFU/ml and 1x104 CFU/ml were applied to determine a threshold level at which 30% of the animals developed meningitis. In a second set of experiments a comparison study between dexamethasone loaded and unloaded silicon rods was performed. For this purpose animals were implanted unilaterally with loaded (n=15) and unloaded silicone rods (n=15), respectively. Five weeks after implantation, animals were infected with a bacterial concentration leading to a 30% meningitis infection as established in the first set of experiments. The development of meningitis within 5 days after infection was assessed by means of documentation of clinical symptoms, analysis of liquor, and histological evaluation of brains and cochlea. The results do not demonstrate a significant difference between the two groups and reveal no enhanced meningitis risk for the dexamethasone-loaded silicone rods used.

Ototoxicity and bacteriostatic activity of methylrosaniline chloride

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PURPOSE: As an empirical treatment of intractable ear discharge, Gentian Violet (GV) has been used in the external ear canal for its excellent antimicrobial and antifungal activity. When GV solution is used in the ear with perforated ear drums, the drug may come in contact with the round window membrane, thus finding a way to the inner ear. To date, however, no report is available on the ototoxicity of GV, except our preliminary reports made in the ARO meetings in February 2008 and 2010. The purpose of this report is to present data on the ototoxic effect of GV that we have evaluated in guinea pigs.

MATERIAL AND METHODS: Using Hartley adult guinea pigs, ototoxicity was evaluated by measuring eighth nerve compound action potentials (CAP) from an electrode on the round window membrane. The stimulus sound consisted of click sounds and tone bursts of 4 and 8 kHz. After the initial CAP measurement, the middle ear cavities of the animals were filled with GV solution, with concentrations of 0.5%, 0.25%, 0.13% or 0.06%. After an interval of 5 minutes, 30 minutes 1 hour, 2 hours, and 24 hours, the middle ear was washed with saline, carefully wicked dry and the reduction in CAP was measured. After all the measurements were completed, the temporal bones were harvested for histopathologic study. Celloidin embedded specimens were cut into 20 micron thick slices and were examined by light microscopy. The bacteriostatic activity of the GV solution against two strains of methicillin-resistant S. aureus, P. aeruginosa, S. pneumoniae, M. catarrahlis, and H. influenzae isolated from ears of patients in our clinic was also studied.

RESULTS: Mild ototoxicity was detected at 30 minutes when using a 0.5% solution, and the same concentration caused reduction in CAP at 60 minutes and a complete abolishment of CAP at 24 hours. When a more diluted 0.13% solution was applied on the round window for only 5 minutes and washed with saline, a severe reduction in CAP was seen at 24 hours. More dilute solutions of GV between 0.125% and 0.06% were effective against all bacteriae tested except for P. aeruginosa. Temporal bones examined 6 weeks after application of 0.5% solution showed severe middle ear inflammation including massive bone formations of the tympanic bulla.

CONCLUSIONS: Although GV has excellent antibacterial and antifungal activity, the use of GV should be limited to the external ear canal. The use of this drug in the middle ear cavity is not recommended.

Cross-defense of auditory hair cells against ototoxicity of gentamicin by non-toxic levels of amikacin

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INTRODUCTION – Amikacin and gentamicin are aminoglycoside antibiotics for treatment of infections in children and adults. Ototoxicity is an important side effect of these drugs resulting in irreversible and bilateral damage to high frequencies. The ototoxicity mechanisms of amikacin and gentamicin are related to stimulation of free radical formation. We used otoacoustic emissions distortion products (DPOEA) and scanning microscopy to verify the action of non-toxic levels of amikacin as a possible protection factor against gentamicin ototoxicity.

OBJECTIVE – On this study we intend to check the possibility of a cross-defense mechanism of outer hair cells protection of toxic levels of gentamicin through a previously period of exposure to non-toxic levels of amikacin.

METHODS – The study was set like this: Group A: 2 guinea pigs, 4 cochleas, water/30 days. Group B: 4 guinea pigs, 8 cochleas, amikacin 20mg/kg/day for 30 days. Group C: 4 guinea pigs, 8 cochleas, gentamicin 160mg/kg/day for 10 days. Group D: 8 guinea pigs, 16 cochleas, amikacin 20mg/kg/day for 30 days followed by gentamicin 160mg/kg/day for 10 days.

RESULTS
Group A – Normal DPOEA, Normal Outer Hair Cells.
Group B – Normal DPOEA, Normal Outer Hair Cells.
Group C – Absent DPOEA, Damage Outer Hair Cells.
Group D – Normal DPOEA, Normal Outer Hair Cells.

We found DPOEA present before and after treatment in group D, as well as normal cilium architecture of outer hair cells on this same group. The results were absent on group C for DPOEA and the cilium architecture was abnormal.

CONCLUSIONS – We conclude that a cross-defense mechanism of outer hair cell occurred by a treatment with amikacin in non-toxic levels before exposure to toxic levels of gentamicin.

Keywords: ototoxicity, aminoglycoside antibiotics, gentamicin, amikacin, cross-defense

Synaptic activity in turtle calyx endings

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A split preparation of the turtle posterior crista allows the visualization of individual calyx endings. Whole-cell recordings are made from the base of calyces, which typically innervate multiple type I hair cells. Fluorescent dye diffusing out of the patch electrode and visualized post-experiment confirms that recordings are made from calyx endings. Resting potentials are typically -70 to -75 mV. Transmembrane resistances (Rm) and capacitances (Cm) are reciprocally related, leading to an estimated time constant of 1.25 ms and a specific membrane resistivity of 125 ohm-cm^2. In voltage clamp, spontaneous synaptic events appear as fast, inward currents (mEPSCs). Consistent with their being mediated by AMPA receptors, mEPSCs are blocked by CNQX and prolonged by cyclothiazide. Quantal rates decline in the presence of Cd^{2+}. Deconvolution is used to detect the start of individual mEPSCs, whose amplitude and timing can then be determined. Inward currents typically average 20 – 60 pA in peak amplitude and show considerable size variability (cv ≈ 0.5). mEPSC shapes of large and small events are virtually identical. Interevent times are exponentially distributed. Spontaneous rates are low (1 mV). A comparison in individual endings shows that mEPSPs can be much larger than predicted from mEPSCs, reflecting amplification due to voltage-gated conductances.

Electrophysiological and anatomical characterization of developing human cristae

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BACKGROUND: Much of what we know about the development and function of the vestibular periphery has come from animal models. However, we know very little about how these animal models compare with humans. Here we describe our preliminary results from physiological and anatomical studies of vestibular hair cells and afferent terminals using in vitro human fetal tissue. Given the temporal differences in vestibular organ maturity between humans and common laboratory animals, it is important to establish a developmental timeline comparing their respective physiology and anatomy. Our goal is to determine the development of whole cell conductances in human hair cells and calyces and those from mice at various developmental stages. This basic information is particularly important if we are to use wildtype and genetically modified mice that adequately represent normal and abnormal human vestibular development and function.

METHODS: Human tissue was collected and prepared according to State legislation and regulatory requirements of the University of Newcastle Human Research Ethics Committee.

PHYSIOLOGY: Inner ears from terminated human fetuses (12 to 18 weeks gestation) were excised and cristae isolated in glycerol-based Ringers' solution. Cristae preparations were then transferred to a recording chamber perfused with L15 cell culture media. Patch-clamp recordings were made from hair cells and calyx afferent terminals that were embedded within the intact cristae using pipettes filled with potassium fluoride internal solution.

ANATOMY: Human tissue (8 to 18 weeks gestation) was fixed in 4% paraformaldehyde for one hour. The tissue was then sectioned and processed for immunohistochemical staining of calcium-binding proteins within the human vestibular epithelium.

RESULTS: We have successfully recorded and labeled a number of human vestibular hair cells that display a variety of inward and outward rectifying conductances. In one example at 14 weeks gestation, an amphora-shaped hair cell (as revealed by intracellular fluorescence), displayed conductances similar to those seen in postnatal mice and rats. This cell however, did not exhibit the negatively activating delayed outward rectifier potassium conductance, gK,L, characteristic of mature type I hair cells. However, it did express a transient current similar to sodium conductances that are typical of immature mouse and rat hair cells. In cristae aged 15 and 16 weeks gestation, we have recorded from four hair cells classified as type II due to their delayed inward and outward rectifier conductances. In contrast to the hair cell recorded at 14 weeks gestation, none of these cells showed evidence of transient sodium channel activation. Interestingly, in some hair cells there appears to be a "collapsing" tail current that is consistent with a transitory accumulation of potassium around the hair cell.

SUMMARY: In this study we provide data on whole cell conductances in developing human cristae. Our preliminary results show evidence that ionic currents in human fetal hair cells are comparable to those recorded in postnatal mouse and rat. At 14 weeks gestation, our results suggest human hair cells are still functionally immature and may undergo distinct changes between 15 and 16 weeks gestational age. Further study will determine whether human vestibular tissue is physiologically mature by 18 weeks gestation.

Damage-induced ERK1/2 activation in mouse utricular organ explants and MDCK monolayers

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Extracellular regulated kinases 1 and 2 (ERK 1/2) are members of the MAPK family that have been shown to be important in both cochlear hair cell death and survival. Short term inhibition (8hr) reduces hair cell death arising from neomycin exposure (Lahne and Gale, 2008), whilst longer term inhibition (24hr) results in increased cochlear hair cell death in both control and gentamicin-exposed groups of neonatal cochlear cultures (Battaglia et. al., 2004). In response to laser or mechanically induced cochlear hair cell damage ERK1/2 activation is localised primarily to the Deiters' and inner phalangeal cells, and spreads out from the damage site in the minutes after damage. Here we examined whether such a pattern of ERK activation is present in mature mouse utricles following damage. Femtosecond pulses from a TiSapphire laser (730nm output) were used to ablate a cluster of 6 hair cells. Immunolabelling for phosphoERK1/2 revealed that hair cell damage triggered ERK1/2 activation in surrounding supporting cells in a pattern analogous to that seen in the immature cochlea. ERK1/2 activation appeared to spread to distances of at least 40 μm from the damage site. We also tested the same laser ablation paradigm in MDCK monolayers, a commonly used model epithelial system. Again, ERK1/2 activitation was seen to extend from the damage site. These data are in agreement with previous work using more severe scrape wounds in MDCK cells (Matsubayashi et. al., 2004). To further investigate the role of ERK1/2 signalling in the MDCK model, monolayers were exposed to a short pulse of UV light (3 minutes) to induce cell death. Fixation at different times post UV exposure revealed a time-dependent activation of ERK1/2. The activation was reduced by the MEK1/2 inhibitor U0126. The numbers of pyknotic nuclei were quantified at predetermined time points post-UV exposure. Data indicate that there was reduced cell death when MEK1/2 were inhibited. Changes in the numbers of actin purse-string rosettes paralleled those of the pyknotic nuclei. We are currently examining the role of ERK1/2 during aminoglycoside induced hair cell death in the mouse utricle. Following 24 hours of aminoglycoside exposure, ERK1/2 activity is observed and appears to be localised to supporting cells, in a similar profile to that following laser induced utricular hair cell damage. We will test the effect of MEK1/2 inhibition on hair cell death in the mature mouse utricle. In conclusion, in mature mammalian hair cell epithelia damage-induced ERK1/2 activation occurs primarily in the supporting cells surrounding damaged hair cells. In addition, we observed similar damage-activated ERK1/2 responses in MDCK monolayers indicating that this may be a useful system in which we can explore this mechanism. GJB is funded by a RNID studentship.

Potassium and calcium concentration in cochlear endolymph after vestibular labyrinth injury

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Partial labyrinthectomy can result in maintenance of hearing under certain circumstances, and the mechanism of the hearing impairment caused by labyrinthectomy is unclear. We hypothesized that disruption of the membranous labyrinth results in electrical leakage and electrolyte imbalance. We investigated the change in cochlear function by measurement of endocochlear potential (EP), [K^+] and [Ca^{2+}] caused by vestibular labyrinth destruction in the acute phase. Hartley guinea pigs underwent lateral semicircular canal (LSCC) transection with suctioning of the perilymph, ampullectomy, or destruction of the LSCC, superior SCC, and lateral part of the vestibule. The EP, [K^+] and [Ca^{2+}] were monitored using double-barreled ion-selective microelectrodes in the second turn of cochlea. The EP showed little to mild change after LSCC transectioning or ampullectomy, but declined variously and drastically after vestibulotomy. The EP did not recover but [K^+] partially recovered after vestibulotomy. Endolymphatic [Ca^{2+}] elevated suddenly but finally normalized after vestibulotomy. [K^+] and [Ca^{2+}] change may have important role in hearing impairment with vestibular destruction.

Injection of virus vector targeting vestibule in mice

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It is known that a lot of children with a congenital deafness have a disorder in vestibular function. We examined the gene transfer to the inner ear using glass tube. In the mouse, three main routes of gene delivery are possible, namely scala media approaches (via a cochleostomy), semicircular canal approaches (via a canalostomy) and round window membrane approaches. In this study, adenovirus (AdV) carrying the green fluorescent protein (GFP) gene were injected in mouse inner ear through the round window and a canalostomy for the purpose of the gene transfer to vestibule. To evaluate the influence of the operation on auditory function and balance function, ABR and the balance test were assessed pre- and post-operatively. Thereafter, transgene expression was observed in the mouse cochlear and vestibular organ. In injection of AdV through the round window, GFP-positive cells were present at the perilymphatic spaces and ampulla. In canalostomy approach, GFP-positive cells were present at perilymphatic space in most samples, and at endolymphatic space in several samples. The signs of vestibular dysfunction were not observed. ABR thresholds did not show any significant changes between before and after the operation of cochleostomy. In this study, gene delivery for vestibular hair cells and supporting cells succeeded in all methods, but injection of adenovirus vector (AdV) via round window resulted in mild hearing loss. On the other hand, injection of adeno-associated virus vector (AAV) via semicircular canal did not result in any hearing loss. In addition, gene expression in vestibular fibrocytes werenot obtained at AdV transfection, though it obtained at AAV transfection. It is suggested that the method using adeno-associated virus via semicircular canal is safety and useful for the purpose of gene transfer to vestibule. To optimize gene delivery, we intend to assess efficiency of each method. The method of this noninvasive transfection into vestibular cells may have a potential to repair balance disorders in human.

Molecular identity and cAMP modulation of the hyperpolarization-activated current in afferent vestibular neurons

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Vestibular afferent neurons of rodents have been shown to express various ionic channels including the hyperpolarization-activated current (Ih) carried by HCN channels. Previous observations indicate that in mouse vestibular ganglion somata blocking the Ih had effect on neither the resting potential nor the action potential morphology (Chabbert et al., 2001). Since HCN channels are regulated by cyclic nucleotides shifting its activation range to more positive potentials and because the molecular identity of HCN channels that originate the Ih current remain unknown, our work was aimed to try to answer those questions. Experiments were made on young and adult Long-Evans rats (P7-10 and P-28). Inner ear was dissected and isolated afferent neurons placed in primary culture. Cultured neurons were recorded with the patch-clamp technique in whole-cell voltage- clamp configuration. In presence of BaCl2, hyperpolarizations beyond -60 mV evoked a time-dependent and slow activating inward nonselective cationic current. The inward current was blocked by extracellular Cs^+ (2 mM, n = 11) or zatebradine (30 µM, n = 9), and potentiated by a high extracellular K^+ concentration (n = 6). These characteristics resemble the hyperpolarization-activated current Ih carried by HCN channels. The activation of Ih started at a potential closed to -60 mV showing a half-maximal activation around -102 mV with a slope factor of 8 mV (n = 24). To explore if Ih is regulated by cyclic nucleotides, cAMP (100 µM) was put into the record pipette (n = 17). In this condition, the Ih current density was increased in significant form and the Ih activation kinetic (tau) was significantly faster than in control group. In the presence of cAMP the Ih half-maximal activation voltage was shifted from the -102 mV to -93 mV without significant changes in the slope factor. In another experimental series the cAMP permeant analogue 8-Br-cAMP (1 mM) was employed. The amplitude of the Ih was potentiated with expositing to 8-Br-cAMP (n = 10) and the tau was faster than in control conditions. In adult rats (n = 22) the Ih current density was higher than the Ih density in young rats, this change was statistically significant, moreover the Ih half-voltage activation was shifted 6 mV toward positive voltages and the tau was faster than in young rats. Using RT-PCR the four HCN subunits were amplified with specific primers. As controls, tissue from brain and heart ventricle was used. The four subunits of HCN were detected in the vestibular ganglia, similar result were obtained from the isolated vestibular epithelium (utricle and semicircular canals). The controls shown, as expected, from the four isoforms of HCN were amplified in rat brain, and only the HCN2 and 4 in heart ventricle. Our results show that the four HCN subunits can originate the Ih current in rat vestibular afferent neurons, most probably forming heteromers. The Ih activation range can be modulated by cAMP shifting it to more functional membrane potential. Finally, through developmental maturation the Ih activation range is shifted to more positive potentials allowing a more relevant participation of HCN channels in membrane potential responses.

Neurofibrillary pathology in the vestibular nuclei of the pR5 mouse

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In patients having suffered from Alzheimer's disease (AD) several brainstem nuclei have been shown to be affected by neurofibrillary changes. Among the vestibular nuclei the lateral vestibular nucleus is known to display many neurofibrillary tangles. From a clinical point of view gait disturbances observed in AD patients may be attributable to malfunctioning of the lateral vestibular nucleus. Functional sequels of this type of pathology, however, are difficult to assess in humans. In this vein, animal models mimicking AD pathology may be suited to serve as experimental targets for functional studies. As a first step a careful comparison between changes in the human versus the mutant murine brainstem is compulsory. We here report about the findings we made in the brainstem of the tangle-forming pR5 mouse by using antibodies against hyperphosphorylated tau-protein. As compared to wild type animals we found an almost selective affection of the lateral vestibular nucleus in the mutant vestibular complex. Numerous AT8-immunopositive neurons were present. Such neurons have been shown to develop into tangle-bearing cells in the course of human AD. The pR5 mouse may represent a suited model to study the consequences of neurofibrillary changes in the lateral vestibular nucleus.

Type 1 allergy-induced endolymphatic hydrops and the suppresive effect of anti-allergy drugs

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OBJECTIVE: To investigate the allergic endolymphatic hydrops and inhibition effect of anti-allergy drugs.

METHODS: Experiment No. 1: Guinea pigs were actively sensitized with DNP-Ascaris twice every month, and were provoked with an injection of DNP-BSA 1 week after the second sensitization. Remaining 10 animals were the control group which received no sensitization but only distilled water. Experiment No. 2: Guinea pigs were actively sensitized in the same manner. One week after the second sensitization. Animals received oral administration of leukotriene antagonist (pranlukast) or histamine H1-antagonist (olopatadine hydrochloride), and were provoked in the same manner 1hr after then. In Experiment 1 and 2, the alterations in the inner ear were investigated histologically at 12, 24, 36 and 48 hrs following provocation, and quantitatively assessed the changes of the endolymphatic space. Experiment No. 3: Expression of leukotriene receptors (CysLT1 and CysLT2) and histamine receptors (H1, H2, and H3) in the endolymphatic sac were investigated immunohistochemistry with ABC method.

RESULTS: Experiment No. 1: Endolymphatic hydrops were observed 12, 24, 36 and 48 hrs after the last sensitization. In all sensitization groups, the increase ratios of the cross-sectional area of the scala media were different significantly from that of the control group ($p < 0.05$). Experiment 2): In animal groups neither with leukotriene antagonist nor with histamine H1-antagonist, endolymphatic hydrops were observed at all. Experiment 3): Cysteinyl leukotriene receptor CysLT1 and histamine H1, H2, and H3 receptors were present in the endolymphatic sac.

CONCLUSION: The sensitization with DNP-Ascaris produced allergic endolymphatic hydrops, and allergic endolymphatic hydrops were inhibited by leukotriene antagonist and histamine H1-antagonists.

Expression of the proinflammatory cytokines in cochlear explant cultures: influence of normoxia and hypoxia

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Hearing loss can be induced by a variety of factors including hypoxia and inflammation. Here, we investigated in vitro the effect of hypoxia on the expression of proinflammatory cytokines in the explanted cochlear tissues. Using RT-PCR, we determined the expression of genes encoding IL-1 beta, IL-6 and TNF-alpha in the organ of Corti (OC), modiolus (MOD) and stria vascularis together with spiral ligament (SV+SL). In addition, using ELISA, we determined the concentration of IL-1 beta and IL-6 in the supernatants of explant cultures. We found that the dissection, explanting and consecutive 24-h normoxic culture results in highly increased expression of IL-1 beta and IL-6, as compared to the freshly isolated tissues. TNF-alpha was upregulated only in the MOD. Interestingly, 24 h of hypoxia decreased the number of mRNA encoding IL-1 beta and IL-6 and increased the number of mRNA encoding TNF-alpha in the SV+SL as compared to normoxia. The concentration of IL-6 measured in the explant tissue culture supernatants was significantly lower in hypoxic than in the normoxic cultures. Our results show that tissue dissection and explanting as well as hypoxia can influence the expression and secretion of proinflammatory cytokines. This implies the presence of tissue-specific regulatory pathways between hypoxia and inflammation in the inner ear.

Detection of drugs in cochlear fluids following round window membrane application in the rat

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The treatment of sudden hearing loss and tinnitus by local delivery of drugs to the cochlear round window (RW) has become a preferential method. By local application, drugs can be delivered in much higher concentrations than systemically and side effects or damaged in other organs can be minimized. Through the last decade substantial evidence has accumulated about the pharmacokinetic in cochlear fluids and predictive models have been introduced that describe the drug allocation in cochlear fluids based on morphometrical and pharmacological studies (Salt & Plontke 2009, Hahn et al. 2006, Plontke et al. 2004). Here, we present data for the intrusion of distinct peptides and drug compounds into the cochlear fluids of the rat after local application to the RW using a gelfoam carrier. Cochlear fluids were taken by micropipettes in vivo and ex vivo, blotted to a nylon membrane and the drugs detected by antibody staining. Immunoblot results will be correlated to functional hearing tests and related to the presumptive drug concentration in the respective compartments in the cochlea. The constraints of local drug application to the cochlea via the RWM in rats will be discussed.

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Intracochlear distribution of intratympanically injected quantum dot-dexamethasone nanocomplex and analysis of the hearing recovery according to the frequency in sudden sensorineural hearing loss

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BACKGROUND: Intratympanic dexamethasone (IT-DEX) injection for treatment of sudden idiopathic sensorineural hearing loss (SSNHL) is a useful treatment option for patients that fail systemic steroid therapy. Intracochlear distribution of the injected steroid varies according to the region of the cochlea injected. Consequently, high concentrations in the basal turn and low concentrations in apical turn have been reported. The clinical results of patients with SSNHL treated with IT-DEX are not always consistent with the above so called apical-basal gradient pattern. There have been no reports on a tendency for improved hearing gains at high frequencies (basal turn). Therefore, we investigated the distribution pattern of dexamethasone in the cochlea and analyzed the results of IT-DEX treatment in patient with SSNHL according to hearing frequencies.

METHODS: The Quantum Dot-Dexamethasone Nanocomplex (QDDN) was injected intratympanically into the bulla of Sprague-Dawley rats and the distribution of the QDDN in the cochlea was analyzed under the fluorescent microscope. One hundred and thirty seven patients with SSNHL were divided into three groups and treated with systemic steroids (N=60), systemic steroids + IT-DEX (N=60), and IT-DEX only (N=17).

RESULTS: The QDDN was distributed throughout the entire cochlea in 1 hour and remained for 24 hours. The highest concentration of QDDN was noted at the spiral ganglion, spiral ligament, and organ of Corti especially in at the basal and middle turn of the cochlea. Distribution of the QDDN paralleled the locations of the glucocorticoid receptors. The hearing recovery at 8000Hz was better in the systemic steroid + IT-DEX group than the systemic steroid only group; however, this difference was not statistically significant.

CONCLUSIONS: Dexamethasone rapidly travels from the middle ear into the inner ear. QDDN appears to be a good vehicle for delivering steroids into the cochlea. IT-DEX injection effectively improves hearing in patients with SSNHL especially at 8000Hz (basal turn), and is appropriate for the distribution of medications in the cochlea from the middle ear through the round window membrane.

Increase of the neuroprotective effect of rolipram on spiral ganglion cells via nanoparticles carriage

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Sensorineural hearing loss, generally caused by degradation of hair cells, leads to a progressive degeneration of the spiral ganglion cells (SGC). The density of healthy SGC must be kept on the highest possible level to assure high efficiency of cochlear implants. A large number of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), have the potential to protect SGC after hair cell loss. Even though many application methods are under investigation, none of them is currently used in clinical settings. We hypothetize, that multifunctional nanoparticles (MFNP) like lipidic nanocapsules (LNC) can be used as biodegradable, cell specific drug carriers to increase the efficacy of conventional application methods. Due to their large molecular weight, most neurotrophic factors are not suitable for incorporation in MFNP. Hence, Rolipram, a phosphodiesterase inhibitor with proven neuroprotective effects on injured spinal cord, was chosen for encapsulation due to its small molecular size. The present study was designed to examine the biological effects of rolipram and encapsulated rolipram on SGC in vitro. Spiral ganglia were isolated from 3-5 day old rats, digested with HBSS including trypsin and DNaseI gently triturated to isolate SGC. Cells were seeded at a density of 1.5 x 104 cells per well of a 96-well plate. The SGC are cultivated in Panserin based media in four groups of LNC, LNC with rolipram, and rolipram or BDNF (50 ng/ml) as positive control. Rolipram was used in concentrations of 38 ng (1:50 dilution), 19 ng (1:100 dilution), 9,5 ng (1:200 dilution) or 6,3 ng (1:300 dilution) per 0,1 ml. Stock solution of LNC contains 4.2 x 1015 FITC labelled LNC per 1 ml. After 48 hours incubation time the cells were fixed and stained by using a neurofilament 200-kD antibody. The groups were compared by the survival rate, neurite outgrowth and soma diameter of SGC. Results showed that rolipram has a positive effect on SGC survival compared with the negative control without much difference between the concentrations. When using encapsulated rolipram, a significantly higher survival rate was found in the 1:100 dilution, whereas the 1:50 dilution showed a significant lower survival rate. Compared to the rolipram and LNC rolipram groups, treatment with BDNF resulted in a significantly higher survival rate. Neurite outgrowth was best in the LNC rolipram group (1:100 dilution). Measurements of the soma diameters showed no significant difference between all groups and concentrations. Our results so far indicate that rolipram has an effect on the neurite outgrowth of isolated SGC that is comparable to BDNF in a concentration of 50 ng/ml. Therefore rolipram might be another promising neuroprotective factor for treatment of SGC. Incorporation of rolipram in LNC is beneficial for the effect on cultured SGC. Therefore also LNC nanoparticles seem to be a very promising drug delivery system to increase the drugs biological effect.

Nanoparticle-silicone composites for reduction of fibrous tissue growth

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Cochlear implants are the method of choice for severely hearing impaired people. Even though many patients can use the telephone again, there is still huge room for improvement especially at the electrode nerve interface. Following implantation, fibrous tissue develops on the surface of the electrode array. This leads to higher impedances and can decrease the performance with the implant. Currently there are several strategies under development for optimization of the interface by reduction of tissue growth. Antimicrobial and antiproliferative effects of metal ions are known from the literature. Previous studies investigated different concentrations of metal salts for their effects on fibroblasts and freshly isolated spiral ganglion cells (SGC). Silver, copper and zinc ions were found to be most promising to reduce fibroblast growth without toxic effects on SGC. In this study nanoparticles (NP) of these metals were incorporated in silicone at concentrations between 0.1 and 3 wt%. Release of metal ions is expected due to corrosion of NP in the silicone body. These nanoparticle silicone composites were again investigated for the influence on fibroblasts. Cells were cultured for 48 h on the materials. Survival of fibroblasts was studied by neutral red uptake and compared to controls without NP. So far a reproducible reduction of cell growth or survival on the NP-silicone composites was not found. Possible reasons might be the larger volume in a culture well compared to earlier measurements of the release kinetics of ions from the composite or also that the high protein content of the cell culture medium causes complex formations.

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Intracochlear distribution of hyperbranched poly-L-lysine after intratympanic application in mice

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Poly-L-lysine represents promising alternative for delivery of drugs or genes to the inner ear structures. In our experiment hyperbranched form of poly-L-lysine (HBPL), molecular weight 13000 g/mol, labeled with fluorhophore (fluorescein-NHS) was used. The aim of the present experiment was to study and compare HBPL intracochlear distribution after the round window (RW) or transtympanic (TT) applications in mice.

Experiments were performed in young C3H mice. Ketamine (35 mg/kg) and xylazine (6 mg/kg) anaesthesia was used during the surgery. Animals were divided into two groups. In the first group 2 µl of HBPL suspension was applied on a small piece of gel foam placed on the RW using postauricular surgical approach. In the second group the animals were injected with 10 µl HBPL suspension through the eardrum (TT application). Animals were sacrificed 1, 3, 6, 11 and 24 hours after the treatment, cochleas were processed and cut and slices were examined with a confocal microscope. Polylysine showed high affinity to bone. One hour after the treatment HBPL were detected in the bony cochlea and later on HBPL spread to the inner cochlear tissues. Slices taken from the cochleae 3, 6, 11 and 24 hours after the application showed HBPL in the organ of Corti, spiral ganglion, basilar membrane, spiral limb and spiral ligament. The time course of HBPL spreading into individual cochlear turns differed for the RW or TT application. After RW application HBPL were detected first in the basal cochlear partitions, whereas TT application enabled HBPL to appear in both scala tympani and vestibuli of the apical turn as soon as 6 hours post-treatment.

We suggest that the principal permeation of HBPL to the cochlea in the case of the RW application is through the RW membrane and stapedial ligament fibers in the oval window (as confirmed in our histological sections), whereas TT application produces a spread of HBPL solution along the whole cochlear surface, absorption to the cochlear bone and penetration to all cochlear turns. The results demonstrate that HBPL can penetrate to the cochlea from the middle ear through the RW membrane, annular stapedial ligament and bony wall of the inner ear. TT application appears to be more suitable when a fast effect is needed.

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The effect of topical sodium thiosulfate in experimentally induced myringosclerosis

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OBJECTIVE: The purpose of this study was to investigate the effect of topical sodium thiosulfate in experimentally induced myringosclerosis.

SUBJECTS AND METHODS: Thirty Wistar albino rats were bilaterally myringotomized. The right ears were treated with topical sodium thiosulfate (STS) or saline daily, and the left ears were left untreated and used as controls. The tympanic membranes were observed by otoendoscopy weekly and tympanometric measurements were performed. All animals were histopathologically examined for myringosclerotic plaques.

RESULTS: Under oto-endoscopy, myringosclerosis were observed around the handle of malleus and near the annular region. The numbers of myringosclerotic ears were significantly more frequent in control and saline groups compared with the STS group ($p<0.05$) and the formations of MS were more severe in control and saline groups compared with STS group ($p<0.05$). Using tympanometric measurement, significantly reduced magnitudes of maximum admittance were observed in control and saline groups compared to normal and STS groups ($p<0.05$). Under histopathologic examination, the tympanic membrane of the STS group appeared thinner than the control group ($p<0.05$), with reduced calcium deposition than control and saline groups.

CONCLUSION: Our results show that sodium thiosulfate has a preventive role in the development of myringosclerosis in the experimental animal model.

Analysis of the head related impulse response by means of an auditory model

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Sound wave entering ears is influenced by the reflections from body, head and pinna. All this, together with interaural differences (ITD and ILD), allows localization of the sound source. The whole process can be described by the Head Related Impulse Response (HRIR). Our paper is focused on localization in “cone of confusion” (the angles with the same ITD and ILD). Since the ITD and ILD are the same, the localization is determined only by the changes of spectrum of stimuli caused by the reflections. Our aim is to analyze differences in sound coming from various directions within the cone of confusion. The results could be used for simplifying of the procedure of virtual auditory space creation. For comparison of the signals a monaural auditory model was used. Two same sound signals coming from different directions within the cone of confusion were independently processed by the auditory model and the linear cross correlation coefficients of the model outputs were calculated. The auditory model is composed of outer and middle ear stage (linear phase, finite impulse response filter along Recommendation ITU-R BS.1387-1), an inner ear stage (cochlear filtration and hair cells transduction) and a neural stage (modulation filterbank). The latter two stages were taken from Jepsen et al A computational model of human auditory signal processing and perception J. Acoust. Soc. Am. 124 (1) July 2008. HRIRs of three human subjects (CIPIC HRTF database) were used. Noise and transient signals were used as stimuli. Frequency regions most affected by the HRIRs were obtained from the result. No significant differences between subject and used stimuli were observed.

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Calculation of the traveling wave velocity – effects on sound coding

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INTRODUCTION: The cochlear traveling wave is amplified by an active system. The passive properties of the basilar membrane stiffness introduce coarse frequency tuning already in the dead cochlea (1). However, the cochlear amplifier is active and introduces sharpening of tuning, nonlinear gain and compression. The traveling wave therefore represent an active nonlinear wave phenomenon (2).

RESULTS: We calculate numerically the velocity of the traveling wave in both active (live) and passive (dead cochlea) cases. We compare this velocity and latency to experimental values in dead cochleas (5) and in normal hearing subjects (3), in subjects with hearing loss (4) and in mammals other than humans.

CONCLUSION: The latencies of the traveling wave at different frequencies have consequences for sound encoding in the spike train of the auditory nerve. When hearing is restored by electrically stimulating the auditory nerve by cochlear implants, the traveling wave latency should be taken into account.

(1) Greenwood D, J Acoust Soc Am, 33: 1344-1356, (1961). (2) Duke T and Julicher F, Phys Rev Lett, 90(15): 158101(1-4), (2003). (3) Donaldson G and Ruth R, J Acoust Soc Am, 93: 940-951, (1993). (4) Donaldson G and Ruth R, J Speech Lang Hear Res, 39(3): 534-545, (1996). (5) Bekesy G, J Acoust Soc Am, 35: 588-601, (1963).

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Biological applications to the acoustic-wave hearing's model

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Biophysical interpretation of the acoustic-wave hearing's model, explaining many hearing phenomena, makes possible to solve common theoretical and practical problems of biology and medicine. Some parameters of the inner ear can be calculated with its help. It's well-known that the hearing of different people have different threshold of the perception of sound higher frequencies. It can be supposed that the solution of this phenomenon is in the difference of cochlea duct length in different people. Acoustic-wave model allows to establish the link between the length Lr of the real cochlea duct and the higher border sound frequency fmax perceiving by sensor cells on the basilar plate: Lr=Lo22log(fmax/fmo). This equation can serve as a mathematics model of calculation of the length of the real cochlea duct of the human's inner ear and its structures. The peculiarity of the human's hearing is the presence of the deviations and disturbance of the perception of the sound frequency characteristics. Distribution of the coordinates of hearing receptors, which do not perceive this or that frequency fd (or frequency rate) as x(fd) can serve as a mathematics model of the localization of the coordinates of the defective sensor cells on the basilar plate when it is being counted out from the basilar area of the cochlea duct. The last ratio can be used as a model of calculating of the measuring of the cochlea duct defective area (basilar plate) non capable to the sound perception at rate Δfd=fd1÷fd2. It rises not only the informative usage of the model itself but based on it quantity method of non-invasive calculation of the biological parameters of the cochlea duct of the human's inner ear and its structures.

Acoustic-wave model of hearing at the pre-receptor level

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Hearing receptors in the human's inner ear are distant according to their nature and regulated according to location. Sound energy undergoes a number of intermediate changes on each structure before cause the receptor's irritation for their transduction into the hearing. Sound effect is born by the oscillation of the vestibular membrane. They produce longitudinal wave in the vestibular scale and diametrical one in the vestibular membrane. Reaching the apex of the cochlea duct the wave experiences reflection. The waves experience dispersion on the vestibular membrane. Dispergirated straight and reflected waves interfere and form a standing wave. It can be shown that its equation – $S(f,x,t)=2Socosk(x–Lo(1–22\log(f/fmo)))\cos\omega((t–Lo(1+22\log(f/fmo))/v(f))$, where x and t are axis and time coordinates, Lo – standard length of the duct, So, f and $\omega=2\pi f$ amplitude, frequency and cyclic frequency, k – wave number, v(f) – speed of the dispergirated waves, fmo= 20 kHz – frequency perceived by the human ear in maximum. Maxims for axis coordinates $xmax(f)=Lo(1–22\log(f/fmo))$, for time – $tmax(f)=Lo(1+\delta(f))/v(f)+n/f$, where n=0,1,2... – is a number of time maxims. The deformity of the standing wave of the vestibular membrane changes sound field of the middle scale. Its affect on tectorial membrane excites sensor cells on the basilar plate in the coordinate xmax(f). Processes taking place in cochlea, being the total sum of biophysical mechanisms realizing hearing on the pre-receptor level, compose acoustic-wave model. It is based on the anatomic- histological functions and on the results of classic experiments.

3-D modeling of cochlear fluid chambers for mechanical simulation models

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A correct geometrical 3-D model of the cochlea is a necessary prerequisite for creating reliable mechanical and fluid dynamical models of the inner ear. Conventional invasive methods for investigating intracochlear structures, such as histology, return insufficient results from later 3-D reconstruction. This is due to distortion which always occur at slice edges during the mechanical cutting of the specimen. Models build upon these data contain inadequacies whose size is impossible to estimate. This is even further severed by the requirement for the smoothing of the resulting model. Non-invasive investigation methods, e.g. computer tomography, are not subject to these obstacles. However, the use of these methods is also complicated due to the difficulty of simultaneous imaging of bony and soft tissue and the presence of digital noise. These obstacles could be surpassed by methods of preparation, imaging and image processing which were developed by the authors and applied to the cochlea. This allowed not only to produce a distortion-free model of cochlear chambers, but also produce a result which is suitable for subsequent use in simulational software. Two isolated cochlea (guinea pig and human) were fixed and stained with OsO4 and 11% iodine solution. Subsequently, they were recorded by means of micro computer tomography (μCT). The resulting image stacks were denoised. The scalae were segmented used own innovative algorithms which allowed the necessary precision in rendering the chambers of the cochlea. The resulting segmentations were visualized using “Amira“. They were also exported into a format which is suitable for mechanical simulational software (e.g. ANSYS). In guinea pig, every of the three scalae could be segmented completely. In human cochlea, the scala tympani has been segmented. These reliable segmentations were obtained despite the complicated spatial geometry of the cochlear chambers. The mechanical and fluid dynamical models of the cochlea based on these geometrical models can be later used for the simulation of fluid-structure interactions under physiological and pathophysiological conditions. However, the geometrical models and visualizations could contribute to the development of new kinds of cochlear implants or other therapeutical innovations in the inner ear.

Altered basilar artery permeability in response to cochlear applied capsaicin: A primary sensory innervation connecting headache and inner ear dysfunction

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Trigeminal neurogenic inflammation is one explanation for the development of vascular headaches. The triggers for this pain are not well understood, but are probably vasoactive components acting on the blood vessel wall. The migraine-related inner ear symptoms of phonopobia, tinnitus, fluctuation in hearing perception, and increased noise sensitivity provide indirect evidence for a connection to basilar artery migraine. The aim of this study was to determine if a physiological basis for neurogenic inflammation exists between cochlear and the basilar artery. The orthodromic activation of sensory fibers of the cochlea was hypothesized to cause neuronally mediated permeability changes in a basilar artery. This hypothesis was tested by activation of putative sensory nerves, to the cochlea with capsaicin. Capsaicin induced neurogenic inflammation induced plasma extravasation in the basilar artery, as observed by colloidal silver leakage with darkfield microscopy and Evans Blue extravasation by laser-scanning confocal microscopy. Cochlear vascular permeability changes were also determined by spectrophotometric measurement of Evans Blue following capsaicin application to the in round window membrane. The capsaicin application also caused a dose and time dependent permeability increase both in the basilar artery and the anterior inferior cerebellar artery (AICA). The most marked extravasation occurred with 0.01% capsaicin, but the effect was also significant with 0.001%, 0.1%, and 1% capsaicin. Sixty minutes following capsaicin Evans Blue extravasation occurred the internal elastic membrane and external elastic membrane of basilar artery and AICA. The colormetric determination of cohlear Evans Blue extravasation also showed significant quantitative differences between the treated, contralateral and control groups. These results characterize a functional connection between the cochlea and vertebro-basilar system through the capsaicin sensitive primary sensory neurons. We propose that vertigo, tinnitus, and hearing deficits associated with migraine arise by excitation of the trigeminal ganglion and plasma extravasation. Cochlear dysfunction may also can trigger basilar and cluster headache.

The role of 5‘ adenosine monophosphate-activated protein kinase (AMPK) in the inner ear

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AMP-activated protein kinase (AMPK), mainly phosphorylates and regulates in vivo hydroxymethylglutraryl-CoA reductase and acetyl-CoA carboxylase (key regulatory enzymes of sterol synthesis and fatty acid synthesis, respectively). The kinase is activated by high AMP and low ATP via a complex mechanism, which involves allosteric regulation, promotion of phosphorylation by an upstream protein kinase (AMPK kinase), and inhibition of dephosphorylation. It is thought of, that the AMPK system protects individual cells by acting as a ‘low-fuel warning system’, being switched on by depletion of ATP. Once activated, it initiates energy-saving measures, and switches on reserve ATP-generating systems (Hardie, Eur. J. Biochem, 1997). We investigated the role of AMPK in the inner ear by applying acoustic overstimulation to AMPK knock out / wild type mice and observing their hearing function during the recovery process via auditory brainstem responses and immunohistological analysis. The results of the ongoing study will be presented and discussed.

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Interaction partners of otoferlin play a role in endocytosis

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Otoferlin has been proposed to be the Ca^{2+}-sensor in hair cell exocytosis, compensating for the classical synaptic fusion proteins synaptotagmin-1 and synaptotagmin-2 (Roux et al. Cell 2006). Yeast two-hybrid assays reveal myosin VI as an otoferlin binding partner (Heidrych et al. HMG 2009). Co-immunoprecipitation assay and co-expression suggest an interaction of otoferlin with rab8 in supranuclear parts of inner hair cells (IHC; Heidrych et al. HMG 2008), as well as a role of the interaction of otoferlin with myosin VI for a proper maturation of the IHC synapse (Heidrych et al. HMG 2009). Long-term stimulation of IHC that do not express otoferlin showed impaired synaptic vesicle pool replinishment (Johnson et al. Nat Nsci 2010), making the search for yet undiscovered interaction partners crucial to fully understand the role of otoferlin in synaptic transmission of IHCs. In the present study, mass-spectrometry assays revealed several new proteins as putative interaction partners of otoferlin that have been further analysed by RT-PCR, immunohistochemistry and co-immunoprecipitation. We here introduce one candidate for interaction with otoferlin that may help to link endocytosis to otoferlin's suggested role in vesicle replenishment in IHCs.

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Decoupling of bicarbonate transport and charge movement in prestin/SLC26A5

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Prestin in outer hair cells, as well as being the source of electromotility, is a member of an anion-transport family, SLC26. Data suggest that it prestins are capable of transporting selected anions in several systems, but in the mammalian system its operation as an exchanger are less clear. OHCs, however, regulate their intracellular pH by transporting bicarbonate (Ikeda et al, J Physiol 442: 447 1992). Based on its family membership, we hypothesize that prestin may be employed as this bicarbonate chloride antiporter. We have reported experiments to show that prestin can regulate pHi, by transporting bicarbonate, the buffer normally present in extracellular perilymph, against chloride. We have used HEK cells transiently expressing rat prestin linked to ecliptic-pHuorin, a construct was sensitive to pH in the physiological range. The pHluorin indicated pH changes near the membrane and so enhanced the signal-to-noise of the recording. Fluorescence monitoring of the intracellular pH was calibrated by using 10μM nigericin to run-down the plasmamembrane proton gradient. With replacement of an external high chloride, hepes buffered, medium with a low chloride-bicarbonate solution, an increase in pHi was observed after the initial acidification due to CO2 entry. In prestin transfected cells, the recovery of pHi was 4 times faster than in untransfected cells. The results are consistent with an increased transport of bicarbonate by prestin, and can be modelled reliably with simple assumptions about the membrane permeation of the species. Measurement of the transfected cell capacitance indicated that prestin-pHluorin exhibited, as with prestin-GFP, a non-linear capacitance, equivalent to 2.6 x10^5 independent charge 'motors' in the membrane (n=5). In this sample, the peak of the capacitance was at -31mV. Irrespective of the external bicarbonate level the cell capacitance was found to be fitted with comparable parameters, once the correction for the junction potential due to low chloride solutions had been applied. Similar bicarbonate insensitivity of the capacitance fitting parameters was also found in measurements in isolated guinea pig OHCs and in situ mouse OHCs. The results complement data of Bai et al, (Biophys J 96:3179, 2009) who showed that formate and oxalate anion transport and non-linear charge movement can be independently manipulated by prestin mutations. The control of pHi in OHCs thus appears to be under control of prestin in the basolateral membrane while leaving the electromotile properties of the cell distinct in physiological conditions.

Calcium signaling in Kölliker's organ: a study based on the hemicochlea preparation

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Transduction of mechanical stimuli by inner hair cells is controlled by finely tuned intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) changes at the synaptic pole [1]. It has been proposed that spontaneous $[Ca^{2+}]i$ variations in the supporting cells surrounding inner hair cells play a role in the development of the inner hair cell afferent signaling [2, 3]. Novel insight into this potentially intriguing relationship can be obtained by Ca^{2+} imaging experiments in the hemicochlea preparation [4]. We report that sustained spontaneous Ca^{2+} activity in neonatal (P6-P7) mouse supporting cells in Kölliker's organ is sensitive to extracellular ATP in the nanomolar concentration range. Furthermore, we show that intercellular Ca^{2+} waves originate in Kölliker's organ interior and spread towards its surface. This approach is of potential general interest for the study of developmentally regulated processes in wild type as well as mutant mouse models.

1. Glowatzki E, Grant L, Fuchs P. (2008) Hair cell afferent synapses. Curr Opin Neurobiol, 18, 389-95.

2. Tritsch NX, Yi E, Gale JE, Glowatzki E, Bergles DE. (2007) The origin of spontaneous activity in the developing auditory system. Nature, 450, 50-5.

3. Tritsch NX, Bergles DE. (2010) Developmental regulation of spontaneous activity in the Mammalian cochlea. J Neurosci, 30, 1539-50.

4. Edge RM, Evans BN, Pearce M, Richter CP, Hu X, Dallos P. (1998) Morphology of the unfixed cochlea. Hear Res, 124, 1-16.

A computational framework to analyze intracellular calcium oscillations and intercellular calcium waves in cochlear supporting cells

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Ca^{2+} is an ubiquitous intracellular messenger that regulates various cellular mechanisms. In the mammalian cochlea, recent work has shown that two mechanisms are responsible for the cell to cell propagation of Ca^{2+} signals:1) diffusion of Ca^{2+} mobilizing second messenger IP3 between neighboring cells, and 2) diffusion of ATP, released from the endolymphatic surface of cochlear supporting cells, which activates P2Y receptors and elicits PLC-dependent generation of IP3 [1-4]. Connexins play a crucial role in both mechanism, as they form both gap junctions channels and hemichannels. To ground the analysis of these phenomena on a quantitative basis, we developed a computational framework, based on the DeYoung-Keizer model for the IP3 receptor [5], comprising the main processes of $[Ca^{2+}]$ regulation in the cytosol: Ca^{2+} extrusion from the endoplasmatic reticulum mediated by IP3 receptors, Ca^{2+} uptake by SERCA pumps, IP3 diffusion between cells through gap junctions, ATP release through connexin hemichannels and diffusion in the extracellular media, Ca^{2+} buffering by mitochondria, regulation of metabotropic receptors activity and IP3 production. The framework uses a realistic supporting cells morphology and topology to investigate how the above mechanisms interact to generate the wide spectrum of Ca^{2+} signals observed under different experimental conditions, from single puffs to regenerative Ca^{2+} waves. We show that, using the extracellular ATP concentration as a bifurcation parameter, the model undergoes a Hopf bifurcation and we conclude that a necessary condition to ensure sustained oscillation of the free $[Ca^{2+}]$ in the cytosol is that extracellular [ATP] remains in a well defined range.

1. Gale JE, Piazza V, Ciubotaru CD, Mammano F. (2004) A mechanism for sensing noise damage in the inner ear. Curr Biol, 14, 526-9.

2. Beltramello M, Piazza V, Bukauskas FF, Pozzan T, Mammano F. (2005) Impaired permeability to Ins(1,4,5)P3 in a mutant connexin underlies recessive hereditary deafness. Nat Cell Biol, 7, 63-9.

3. Piazza V, Ciubotaru CD, Gale JE, Mammano F. (2007) Purinergic signalling and intercellular Ca^{2+} wave propagation in the organ of Corti. Cell Calcium, 41, 77-86.

4. Anselmi F, Hernandez VH, Crispino G, Seydel A, Ortolano S, Roper SD, Kessaris N, Richardson W, Rickheit G, Filippov MA, Monyer H, Mammano F. (2008) ATP release through connexin hemichannels and gap junction transfer of second messengers propagate Ca^{2+} signals across the inner ear. Proc Natl Acad Sci U S A, 105, 18770-5.

5. De Young GW, Keizer J. (1992) A single-pool inositol 1,4,5-trisphosphate-receptor-based model for agonist-stimulated oscillations in Ca^{2+} concentration. Proc Natl Acad Sci U S A, 89, 9895-9.

Simultaneous calcium entry sites in cochlear inner hair cells of the adult mouse imaged by 2-photon confocal microscopy

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Each inner hair cell (IHC) of the mammalian cochlea contains a cluster of 10-20 ribbon synapses located on lower region of the basolateral membrane. In vivo recording of auditory nerve fibres show that not all fibres have the same thresholds for sound (e.g Taberner and Liberman, J Neurophysiol 93:557-69. 2005) suggesting that there may be local differences between IHC synapses. We have investigated presynaptic variations between the sites in the adult wild type mouse by using simultaneous whole cell recording and calcium imaging in IHCs using a preparation of the organ of Corti of adult (P21-P60) mice. The organ of Corti remains in the excised temporal bone and it is bathed in normal perilymph. The preparation preserves orientation and the cellular environment of the IHCs. All procedures were carried out in accordance with local UK Home Office guidelines. A small opening made in the apical cochlea allowed access for patch recording pipettes and for imaging the basal synaptic pole of the cell using a 2 photon laser scanning confocal microscope. With a 63x NA 1.0 WI objective, the resolution in the XY-plane was 300 nm and the resolution along the Z-axis was 1.5 um. Calcium indicators, either OGB-1 or OGB-5N were included in the pipette, and were excited at 935 nm (or transilluminated at 830 nm for pipette guidance). Pipettes contained caesium to block all but 1-2 nA of the large outward potassium currents present at 0mV revealing, at room temperature, a small (typically 80 pA) inward Ca current, peaking at -20 mV. A maximum imaging frame rate of 70 Hz was achievable on this system. On cell depolarization, the signal from the calcium indicator increased at highly localised sites at the base of the IHC. The typical mean diameter of the such ‘hotspots’ was 0.65 µm, suggesting a calcium entry area of no more than 0.5 µm in diameter. Depolarization of the IHC for 100 ms from -70 to -20 mV elicited a rapid rise of a signal and decay over approximately 200 ms. When the recording plane was optimally positioned, the signal increased to a local sustained peak. By choosing imaging planes close to the basal pole of the IHC up to to six localised ‘hotspots‘ could be detected in one section. Such numbers are consistent with the number of ribbon determined by immunohistochemistry (Meyer et al, Nat Neurosci 12:444-53, 2009). Although cell responses could be variable, the peak amplitude of the responses differed between distinct regions of a single IHC. Normalised with respect to background, responses recorded from the modiolar side of the IHC base (n=29 ‘hotspots‘) were 1.34 times larger than from the abneural side (n=17 ‘hotspots‘). There was no significant difference between the amplitudes of fluorescence responses located on either side of the IHC along the longitudinal cochlear axis. The results support the hypothesis that variation in organised presynaptic calcium control between ribbon synapses within a single IHC may determine the differential sensitivity of and release of neurotransmitter to individual auditory nerve fibres.

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Systematic effects of interference tones presented above f2 on DPOAE residuals

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Recently, DPOAE level/phase (L/P) maps were acquired from normal-hearing humans (Martin et al. J Acoust Soc Am 125:EL85-92, 2009) and normal and noise-damaged rabbit ears (Martin et al. J Acoust Soc Am 127:1955-72, 2010). These L/P maps were collected with and without an interference tone (IT) placed either 44 Hz below the DPOAE frequency (fdp) or at 1/3-octave (0.33 oct) above f2. The IT was presented on alternate trials to minimize time-dependent changes, and vector differences were computed between the two conditions to obtain a residual. With the IT 0.33 oct above f2, the residual indicated that DPOAE components generated at, or basal to the IT frequency place, comprised a large part of the ear-canal signal in both species. It was suggested that these DPOAE components were part of a distributed source of DPOAEs generated by interactions between the tails of the primary-tone traveling waves (TWs). If this hypothesis was true, then it would be expected that the size of the residual revealed by the IT would vary systematically with primary-tone level and with the distance the IT was placed above the f2 primary-tone. To test this notion, DP-grams were obtained in normal rabbit ears (f2 from 0.5-20 kHz) for equilevel (L1=L2=45; 50; 55; 60; 65; 70; 75 dB SPL) and unequal-level (L1,L2=45,25; 50,35; 55,45; 60,55 dB SPL) primary-tone level series. Each series was collected with the IT varying between 45-75 dB SPL in 5-dB steps. This protocol yielded 49 DP-grams for the equilevel and 28 DP-grams for the unequal-level series. All level-series settings were repeated with the IT placed at 0.33, 0.5, 0.66, 1.1, 1.5, and 2 octaves above f2. DP-grams with and without the IT and the residual (vector difference) were plotted on the same axis for comparison. For equilevel series measured with high-level 0.33-oct ITs, large residuals were obtained, and there was no indication that the IT affected the largest emission components around f2 in that DP-grams with/without the IT were essentially identical. This was not the case for some unequal-level primary tones where the high-level ITs, with respect to the primaries, lowered the overall level of the DP-grams possibly by affecting near-f2 components, and thus this interaction could have contributed to the residual. However, in all cases, ITs placed 1.1 to 1.5-oct or more above f2 clearly yielded smaller residuals than those closer to f2, and larger residuals were obtained for higher as compared to lower primary tone levels for these particular IT conditions. These findings support the hypothesis that the residuals revealed by ITs presented above f2 reflect distributed DPOAE components generated by interactions in the tails of the primary-tone TWs, and that these sources diminish as the distance above f2 increases.

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Optical coherence tomography for the diagnosis of inner ear diseases

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Sensorineural hearing loss is one of the most difficult disability to treat. In the inner ear, more specifically in the cochlea, various components including inner/outer hair cells, supporting cells, spiral ganglion neurons, stria vascularis and spiral ligament, are arranged precisely and function cooperatively. Damage of each one of these can cause specific type of inner ear dysfunction, resulting hearing impairment. However, in clinics, it is difficult to identify specific structure that is damaged in the hypofunctioned inner ear with its structure intact. In other words, we treat hearing impairment without recognizing what is to be treated in the inner ear. Eventually, the treatment of inner ear diseases are difficult. Optical coherence tomography (OCT) is a relatively new optical image aquisition modality employing low-coherence interferometry to produce cross-sectional image of optical scattering from internal microstructure. For ophthalmologists, OCT is already an essential diagnostic equipment for the live visualization of the cross-section of retina or cornea, and the special resolution has already reached to 10-μm order that is capable of visualizing small vessels and cells. Clinical application for the diagnosis of skin, blood vessels and teeth has been started. Non-destructive inspection of semiconductor materials is also a good application of OCT imaging. For otologists, the visualization of the intra-cochlear structure of damaged cochlea with its capsule intact should provide diagnostic information of hearing impairment that has never been available so far. In this study, we sought the possibility of OCT for the diagnosis of inner ear diseases. The equipments used were ones that were already commercially available for ophthalmology or dermatology. PFA-fixed cochleae of guinea pigs (Hartley, adult) were removed and scanned with the OCT through a small opening at the apex of the cochlea. Inner structures such as organ of Corti and Reissnel's membrane were visualized, meaning sufficient resolution of current OCT for the visualization of these structures. Next, by scanning through the bony capsule of the cochlea, structures of the lateral wall were visualized. In order to evaluate the function of the structure of stria vascularis, measurement of endocochlear potentials and histological analyses have been necessary. OCT have the possibility to provide diagnostic information of such structures without making any puncture or sections.

Glial cell distribution in the mouse cochlea and in cultured spiral ganglion neurons

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The tissue culture system of spiral ganglion cells with projection of neuronal processes is an established model to study a variety of soluble and insoluble factors. There are many of such guidance cues that determine the length and growth behaviour of spiral ganglion neurites. Only little is known about the effects of the non-neuronal cells like glial cells that are found in close contact and nearby the neurites of spiral ganglion explants. In order to study the effects of neurite-accompanying and co-cultivated cells on spiral ganglion neurites, we have used immunohistochemistry and scanning electronic microscopy in spiral ganglion cell cultures of C57BL/J6 mice. Furthermore, were examined the temporal and spatial distribution of glial cell markers of paraffin sections of the mouse cochlea during the hearing development. Our findings suggest an important role of glial cells in spiral ganglion outgrowth. The results indicate that differentially distributed glial cells in the spiral ganglion tissue culture can control the projection of spiral ganglion neurites, as it was described in cultured retinal ganglion cells and neurons of the central and peripheral nervous system before. Further investigations should also focus on factor-dependent effects on these cells, moreover this culture model provide a basis on cochlear implant research and improvement of neurite-electrode contact as well as regeneration of spiral ganglion neurites.

Cochlear implantation trauma study with two surgical methods and possible hearing protection by Dexamethasone release

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The release of dexamethasone reduces hearing loss due to cochlear implant insertion trauma (Jolly et al 2010). Based on this evidence we evaluated the effects of intratympanic insertion of 10% dexamethasone eluting silicone rods in an animal model. With the purpose of verifying the completeness of cochleosthomy healing after different degrees of trauma, we used two surgical methods using different eluting rods. Six guinea pigs were carefully implanted with soft eluting rods and six controls with non-eluting ones, and five guinea pigs were implanted causing mechanical trauma with wired (stiffer) eluting rods and five controls with non-eluting wired rods. Implantations were performed through a 0.7mm cochleostomy, followed by a 3-mm deep rod insertion. Hearing threshold audiograms were acquired prior to implantation and during the next two/four weeks by recording compound action potentials with electrodes near the round window. After two/four weeks the bulla with the cochlea was removed, decalcified, embedded in paraffin and longitudinally cut into 5-µm thick sections. For each sample we examined the macrophage presence and the Scala Tympani occlusion near the cochleostomy. Rods were explanted and tested for bacterial contamination. Histological results from the atraumatic surgeries showed no appreciable differences between the non-eluting and the 10% dexamethasone eluting rods. The two different surgery approaches in presence or absence of dexamethasone did not show significant immunological response or enhanced number of macrophages. Moreover no bacterial contamination was detected on the implant rods. However, in the presence of 10% dexamethasone eluting rods, the average tissue growth was always lower in comparison to non eluting ones. This supports the use of 10% dexamethasone eluting rods with slow-release as an anti-inflammatory additive in cochlear implants.

Biofunctionalization of cochlear implants – alginates as scaffold for neurotrophic factor producing cells

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BACKGROUND: Sensory-neural hearing loss leads to degeneration of spiral ganglion cells (SGCs). For the beneficial outcome of cochlear implants (CI) the SGC-density plays a decisive roll. Several studies indicate that SGC degeneration can be reduced by brain-derived neurotrophic factor (BDNF). Some in vivo results state that BDNF should be delivered locally to the inner ear for months because a cessation of treatment leads to accelerated neuronal degeneration. Therefore, to be clinically relevant for cochlear implant patients, the protective effect of neurotrophins should persist and the treated SGCs should remain functional.

A permanent growth factor application may be realized via a cell-based drug delivery system e.g. via BDNF producing fibroblasts. Encapsulation of these cells into a scaffold could avoid immune reaction of the recipient, migration, and uncontrolled proliferation of the cells. We hypothesize that ultra high viscous (UHV)-alginate (clinical grade) could be a suitable scaffold for these cells because of its bioinert and semipermeable characteristics.

METHODS: Lentiviral vectors were used to genetically modify a murine fibroblast cell line (NIH3T3 cells) to produce BDNF as well as GFP as a marker gene. These cells were mixed with a brown algae extract and afterwards the mixture was polymerized to a hydrogel by contact to a barium solution. We examined this barium crosslinked UHV-alginate in vitro for its properties to encapsulate the lentivirally modified NIH3T3 fibroblasts producing green fluorescent protein and BDNF for different time periods. The survival rate and proliferation of the cells in the alginate was determined by cell counts of cryosections, the BDNF release out of the alginate-scaffold into the medium was examined by ELISA and the survival rate of dissected SGC cultivated with the BDNF containing medium from the alginate-cell culture was detected.

RESULTS: BDNF producing fibroblasts cultivated in UHV-alginate demonstrate an acceptable survival for up to 30 days. The amount of BDNF in the medium detected by ELISA seems to be low but added to the SGC culture this BDNF, produced from in alginate incorporated fibroblasts, increased the SGC survival in vitro.

CONCLUSION: The combination of alginate and neurotrophic factor producing fibroblasts induced a desired positive biological effect on spiral ganglion cells in vitro. Therefore the UHV-alginate seems to be a suitable scaffold for BDNF producing fibroblasts. UHV-Alginates are a promising material for cochlear implant biofunctionalization but further research has to be done to evaluate the optimal number of incorporated fibroblasts to evoke the maximum SGC protection. Additionally, the technical aspects of how to coat the CI with the cell containing alginate have to be solved.

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Culture model of neurotrophin mediated regulation and stimulation of neurite outgrowth in mouse cochlear spiral ganglia

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The development of cochlea implants (CI) marks a turning point in therapy for deaf or profoundly hearing impaired people. Currently the technique allows stimulating the auditory nerve by 12-24 independent channels. However, these channels are interfering and allow only a reduced spatial resolution. In the normal condition with acoustic stimulation there are 3500 highly selective, frequency specific inner hair cells which stimulate the auditory nerve. The performance of a cochlear implant is dependent on the number of neurons available for stimulation and by the nerve-electrode interaction. The nerve-electrode-interaction may be improved by stimulating the outgrowth of remaining neurites towards and optimally onto the CI. Such a nerve neurite growth stimulation is mediated by neurotrophins as brain derived neurotrophic factor (BDNF), activating TrkB receptors. However, the inhibitory activity of myelin cells, mediated by the low-affinity neurotrophin receptor p75NTR inhibit neurite outgrowth. As a paradox, the stimulatory effect of BDNF is opposed by its simultaneous binding to the low-affinity p75NTR receptor in an inhibitory environment. An organotypic culture model of the mouse spiral ganglion was established (mouse strain NMRI; postnatal day 4-6). Culture slides were coated with different extracellular matrices (ECM) to compare their growth stimulating effect. The neurite outgrowth was analyzed and quantified with AxioVision (Scanning, Stitching), ImageJ (generating binaries, count black pixels) and a custom made program (count neurites, determine neurite length). Stimulation of neurite outgrowth was quantified for BDNF, synthetic, selective TrkB ligands (Williams et al., J Biol Chem. 2005) and a selective Rho-A-kinase-inhibitors (Y27632). Inhibition of neurite outgrowth was assessed for MAG-Fc (myelin-associated glycoprotein), PI3K (phosphoinositide-3-kinase) inhibitors (wortmannin, LY294002), PKA (protein kinase A) inhibitors (H-89, KT7520) and synthetic, selective TrkB antagonists (Williams et al., J Biol Chem. 2005). The extracellular matrix coating laminin/poly-D-lysin provided an optimal surface for neurite outgrowth. Stimulation with BDNF was achieved in a dose and a time dependent manner. Neurite outgrowth was stimulated by the synthetic TrkB ligand. Inhibitors were capable to inhibit these effects in a dose and time dependent manner. MAG-Fc created an inhibitory environment which could be escaped by selective TrkB ligand and which could be compensated by the Rho-A-kinase-inhibitor. The organotypic culture model of the postnatal mouse spiral ganglion is suitable to assess stimulatory and inhibitory compounds. A synthetic, selective TrkB ligand proves to be a potential candidate for selective stimulation of neurite outgrowth. To overcome inhibitory environment, more potent TrkB-agonists are required. Selective inhibition of p75NTR pathway improves neurite outgrowth in an inhibitory environment.

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A new experimental otoprotective protocol against noise induced hearing loss

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BACKGROUND: Studies in the literature have demonstrated that exposure to noise increases the production of free radicals, which damage the inner ear causing hearing loss. Data from animal studies show that antioxidants can compensate against noise-induced stress and sensory hair cell death. The study evaluated the efficacy of two protector drugs i.e. Acuval 400® and Acuval 400 with 500 mg/kg of Coenzyme Q10 terclatrate, against noise damage in a rat animal model.

MATERIAL/METHODS: Sixty Sprague Dawley rats were divided into : A) noise exposed animals; B) animals exposed to noise and treated with Acuval 400; C) animals exposed to noise and treated with the fortified Acuval; D) animals treated only with Coenzyme Q10 terclatrate, and E) not-treated, control animals. The drugs were administered orally 5 times : 24 and 2 hrs prior to noise exposure, and then daily for three days. The auditory function of each animal was assessed by measuring Auditory Brainstem responses (ABR) in the range from 2 to 32 kHz at t= 1, 7, 14 and 21 days after noise exposure.

RESULTS: Group A animals, presented significant mean threshold shifts ($p < 0.001$) at all tested frequencies, 24h after the noise exposure. A possible permanent hearing loss of 25dB on average was noted on the 21st day after noise exposure. The mean threshold shift among the animals in Group C was significantly less than group A on day 1. On day 21, for all frequencies, except 2K, there was no statistically significant differences between the pre (t=0) and 21d responses. Animals in Group D did not show any significant differences in the hearing threshold during the experiment.

CONCLUSION: The data of this study suggest that a fortified Acuval solution, administered orally, protects from Noise Induced Hearing Loss.

Expressions of endothelin-1, endothelin receptor A, B in the cochlea of noise induced transient threshold shift rat model

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Recent research has shown that endothelin and its receptors have important role in blood flow regulation of many organs including cochlear. On the other hands, the causes of noise induced hearing loss are thought of direct mechanical effect and alteration of metabolism after blood flow reducing on cochlear. The aim of this study is to investigate expression of endothelin- 1(ET-1) and endothelin receptor A,B(ETA, ETB) in the cochlear of noise induced transient threshold shift rat model.Normal twelve S-D rat were exposed to noise. Four of them were sacrificed at post-noise 1 day and another four animals were sacrificed at post-noise 4 day.

The remainder four animals were sacrificed at post-noise 7 day. Four were normal controls that were not exposed to noise. Auditory function was evaluated with auditory brainstem responses and the expression of ET-1, ETA,ETB was examined by immunohistochemistry. The transient threshold shift was recovered at post-noise 4 day. Expression of ETA and ETB were changed in the course of time. It is suggested that there is some relation between recovery of noise induced, transient threshold shift and expression of ET-1, ETA, ETB in cochlea.

A proteome of the stria vascularis vasculature and that noise-induced vascular leakage correlates with PKCη regulation of Na^+, K^+-ATPase α1-initiated phosphorylation of occludin

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The integrity of the cochlear blood-labyrinth barrier (BLB) in the stria vascularis (SV) is critically important for maintaining cochlear homeostasis, including the endocochlear potential, which is the essential voltage drive of sensory hair cells. However, the molecular mechanisms that regulate BLB permeability in the inner ear are poorly understood. To provide a broad perspective of these mechanisms, we used a mass-spectrometry, shotgun-proteomics approach to identify proteins purified from stria vascularis capillaries with a novel "sandwich-dissociation'' method. More than 600 proteins wereconfidently identified, including proteins involved in metabolism, ion transport, biological regulation, and signal transduction. The membrane-bound protein, Na^+-K^+-activated adenosine triphosphatase (NKA) α1 subunit was the most abundant protein in the BLB. Additionally, functional studies demonstrated that NKA α1 regulates tight-junction permeability in the BLB. A decrease in enzymatic activity of NKA resulted in hyperphosphorylation of occludin, a tight-junction protein, which in turn increased the BLB permeability. Protein kinase C eta (PKCη) was found to be an essential mediator for NKA α1-initiated phosphorylation of occludin. The noise-induced vascular leakage, assessed by extravagation of serum protein immunoglobulin G (IgG) to the extracellular space in the SV, was significantly attenuated by blockage of PKCη activity. The results presented here provide first evidence that NKA α1 interaction with PKCη and occludin play pivotal roles in the regulation of endothelial barrier functions that control BLB permeability. Furthermore, the data suggested a potential new strategy for treating noise-induced BLB leakage through therapeutic manipulation of NKA α1 and PKCη enzymatic activities.

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Liposome-encapsulated hemoglobin alleviates hearing loss after transient cochlear ischemia and reperfusion

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Acute interruption of the blood supply to the cochlea is thought to be one of the major causes of sudden deafness. We have investigated the effects of transient cochlear ischemia on hearing and the histology of the inner ear in experimental studies with Mongolian gerbils (*Meriones unguiculatus*). The results showed that the ischemia caused hearing loss mainly at higher frequencies, and that the inner hair cells (IHCs) degenerated predominantly at the basal turn. Using this animal model, we have also studied various therapeutic agents for preventing ischemic cochlear damage. Liposome-encapsulated hemoglobin (LEH), which was originally developed as an artificial oxygen carrier, was tested in transient cochlear ischemia and reperfusion as an experimental model of sudden deafness.

METHODS: Mongolian gerbils were randomly assigned to receive 2 ml/kg of either low-affinity LEH (l-LEH, P50O2=40 mmHg), high-affinity LEH (h-LEH, P50O2=10 mmHg), homologous red blood cells (RBCs), or saline (each group n=6) 30 min before 15-min occlusion of the bilateral vertebral arteries and reperfusion. Sequential changes in hearing were assessed by auditory brain response 1, 4, and 7 days after ischemia and reperfusion, when the animals were sacrificed for pathological studies.

RESULTS: h-LEH was significantly more protective than l-LEH in suppressing hearing loss, in contrast to RBC-treatment without any benefits over saline-treatment, over a wide range of auditory frequencies, 8, 16 and 32 kHz, where hearing loss was most severe; 12±6 dB in h-LEH, 26±7 dB in l-LEH, 41±3 dB in saline, 42±8 dB in RBC (P<0.05 between any two groups) on the first day after cochlear ischemia/reperfusion. Thereafter, hearing loss improved gradually in all groups, with a significant difference among groups up to 7 days, when morphological studies revealed that the inner hair cells, but not the outer hair cells, were lost significantly among groups in the same order.

CONCLUSION. The results suggest that pretreatment with h-LEH (2 ml/kg) is significantly more protective than l-LEH in mitigating hearing loss and underlying pathological damage, in contrast to transfusion that is without any benefits over saline-treated controls, 7 days after transient cochlear ischemia and reperfusion.

Simultaneously reduced Na, K-ATPase and NKCC1 expression in murine cochlear lateral wall contributes to conservation of endocochlear potential following a sensorineural hearing loss

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Mechanisms of the response in murine cochlear lateral wall following a sensorineural hearing loss are poorly understood. We focused on comparing the endocochlear potential (EP) with morphological changes in lateral wall and expression of four important potanssium (K^+) transporters, including the α1 and α2 isoforms of Na, K–ATPase, Na-K-2Cl-Cotransporter-1 (NKCC1) and potassium channel KCNQ1, in a mouse model of sensorineural hearing loss induced by co-administration of aminoglycoside and loop diuretic. An interesting responsiveness of EP became evident: EP displayed a significant decline at 12 hours posttreatment followed by completely recovery since 2 days posttreatment and maintained at near nomal levels for a long time up to 112 days posttreatment. Despite this recovery, there was a significant and progressive decrease in strial vascularis thickness, which was predominantly due to atrophy of marginal cells. Meanwhile both protein and mRNA expression of α1,α2 isoforms of Na, K–ATPase and NKCC1 in cochlear lateral wall were dramatically reduced following a longe time recovery but expression of KCNQ1 remained unchanged. These observation provide insight into the detailed mechanisms of EP modulation following a sensorineural hearing loss and may have crucial implications in the future treatment of drug-induced hearing loss.

Spiral ganglion cell morphology in guinea pigs after deafening and neurotrophic treatment

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A healthy condition of the auditory nerve is essential for hearing in patients with a cochlear implant. It is well known that spiral ganglion cells (SGCs) degenerate in hair-cell deprived cochleas. The focus of this study is on size and shape (circularity) of SGCs, which are a measure of cell viability. In addition, the effect of neurotrophic treatment on SGC morphology was investigated. Guinea pigs were deafened by co-administration of kanamycin (400 mg/kg) and furosemide (100 mg/kg). At different points in time after deafening (1, 2, 4 and 8 weeks) and at different cochlear locations the size and circularity of SGCs was measured. To examine the effect of neurotrophic treatment a second group of guinea pigs was first deafened and after 2 weeks the right cochleas were implanted with a drug-delivery cannula. Brain-derived neurotrophic factor (BDNF; 100 µg/ml) was infused into the cochlea over a period of 4 weeks at a rate of 0.25 µl/hr. Immediately after finishing the treatment SGC viability parameters were measured. The left cochleas served as untreated controls. SGC size gradually decreased after deafening in the basal and apical cochlear turns. Already after one week a decrease in size was observed, which was well before the packing density started to decrease. After BDNF treatment survival of SGCs was enhanced, in particular in the basal turn. SGCs had become noticeably larger than normal throughout the cochlea, including the middle and apical turns. We conclude that both after deafening and after neurotrophic treatment a change in size is observed prior to a change in survival. Surviving SGCs in hair-cell-deprived cochleas are smaller than normal, which indicates a reduced viability. An increase in size as observed after neurotrophic treatment might indicate restored viability.

AM-111 protects against hearing loss and inner hair cell loss after transient cochlear ischemia

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Sudden sensorineural hearing loss often results from a disruption of cochlear function that is thought to be caused by an acute interruption of the blood supply. In our previous study, we investigated transient cochlea ischemia-induced hearing loss in gerbils as a model of sudden sensorineural hearing loss and found that inner hair cells (IHCs) are more vulnerable to damage than outer hair cells (OHCs). On the other hand, ischemic insult induces a variety of cytotoxic reactions that are characterized by the production of superoxide anions and nitric oxide. These anions act together on other molecules to produce more toxic free radicals that result in ototoxicity. There stimulate MAPK-JNK cascade, and occur apoptosis of IHCs. The purpose of this study was to investigate protective effects of AM-111, a peptide inhibitor of c-Jun N-terminal kinase, against hearing loss and IHC loss after transient cochlear ischemia. Hearing was assessed by recording the ABR threshold and the rate of IHC loss was assessed in specimens stained with rhodamine-phalloidin and Hoechst 33342. In animals treated with saline, the significant ABR threshold shift and the decrease in the number of IHCs were observed. By contrast, the threshold shift and IHC loss were suppressed in animals treated with AM-111. These results suggest that AM-111 protects against the damage to the inner ear following transient ischemia.

Combination therapy of systemic steroids, antiviral agent, anticoagulants and stellate ganglion block for the treatment of sudden sensorineural hearing loss

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Sudden sensorineural hearing loss(SSNHL) is commonly defined as a loss of at least 30dB in 3 contiguous frequencies occurring within 3 days. Systemic steroids have become the most widely accepted treatment option for SSNHL. As viral infection and vascular compromise have been considered as specific causes of SSNHL, antiviral agent, anticoagulants and stellate ganglion block have been used for the treatment of SSNHL although evidence of their effectiveness is weak. The aim of our study was to evaluate the hearing recovery rate in combination therapy group(systemic steroids, antiviral agent, anticoagulants and stellate ganglion block) as compared with in systemic steroids alone. A total of 85 patients diagnosed with SSNHL were treated with combination therapy (Group A, 46 patients) or systemic steroids only(Group B, 39 patients). Hearing improvement was defined as hearing gain more than slight improvement using Siegel's criteria. All patients were treated with a 10-day course of systemic steroids(60mg prednisolone for 5 days, followed by tapering for 5days). Acyclovir, heparin and stellate ganglion block were included in the treatment regimen of Group A. The overall rate of hearing improvement was 60.9%(28/46 patients) in Group A, which was significantly higher than 38.5%(15/39 patients) in Group B. The distribution of prognostic factors was not statistically different between two groups except degree of initial hearing loss which was severer in Group A. When hearing improvement was analyzed according to the prognostic factors, Group A showed the better recovery rate than Group B in patients with hearing loss more than 70dB, age more than 41yrs, dizziness and early treatment(<1week). The results of this study suggest that SSNHL patients treated with combination therapy have a higher likelihood of hearing improvement than those treated with systemic steroids alone.

Simvastatin and gentamicin-induced auditory hair cell loss

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INTRODUCTION: Inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, known as statins, are commonly used as cholesterol-lowering drugs. Statins act by blocking the enzyme necessary for the production of L-mevalonate, an intermediary product in the synthesis of cholesterol. During the past decade, evidence has emerged that statins also have neuroprotective effects. The effects of statins in the mammalian inner ear are largely unknown. We tested whether simvastatin, a commonly used statin, can protect hair cells from gentamicin-induced damage in vitro.

MATERIALS AND METHODS: Expression of HMG-CoA reductase mRNA in the rat cochlea was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR). Protection of auditory hair cells from gentamicin was tested using two different concentrations (1 μM and 10 μM) of simvastatin and in combination of simvastatin (10 μM) and mevalonate (100 μM).

RESULTS: We detected HMG-CoA reductase mRNA and in the organ of Corti (OC), spiral ganglion, and stria vascularis by RT-PCR. Moreover, we could see significantly less hair cell loss in the OCs that were pretreated with either 1 μM or 10 μM of simvastatin as compared with samples treated with gentamicin alone. The protetive effect of simvastatin on auditory hair cell damage could be reversed by addition of mevalonate 100 μM.

CONCLUSIONS: HMG-CoA reductase mRNA is expressed within the cochlea. Decreased hair cell loss in simvastatin-treated samples that had been exposed to gentamicin provides evidence for a protective effect of simvastatin in amiglycoside-induced hair cell death in vitro. There is evidence that the protective effect of simvastatin is mediated through the mevalonate pathway, since the addition of mevalonate reversed the protective effect of simvastatin in aminoglycoside-induced hair cell death in vitro.

Role of homocysteine metabolism in hearing loss

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Genetic and environmental factors contribute to hearing disorders. Several genetic mutations causing sensorineural hearing loss in humans have been further studied in animal models and the underlying molecular mechanisms of hearing dysfunction uncovered. Thus, betaine-homocysteine methyltransferase (BHMT) expression is decreased in the Connexin-30 null mouse (1), being BHMT one of the enzymes responsible for homocysteine (Hcy) remethylation leading to methionine synthesis (2). Environmental factors such as noise (3, 4) also cause oxidative imbalance in the organ of Corti and the stria vascularis. A consequence of the increase in plasma Hcy levels is the decrease in the production of the antioxidant glutathione that together with an increase in the atherogenic and neurotoxic effects of Hcy constitute an adverse situation for the function of the stria vascularis, and as a consequence, hearing function may result compromised. Dietetic and nutritional factors can favourably modulate the metabolism of Hcy. Here, we have studied the role of Hcy metabolism in hearing physiopathology in order to asses the usefulness of BHMT and Hcy metabolites as early molecular indicators of hearing damage in different animal models (3-5).

1. Cohen-Salmon, M. et al. (2007) Connexin30 deficiency causes instrastrial fluid-blood barrier disruption within the cochlear stria vascularis. Proc Natl Acad Sci USA 104: 6229.

2. Pajares, M.A. and Pérez-Sala D. (2006) Betaine homocysteine S-methyltransferase: just a regulator of homocysteine metabolism? Cell Mol Life Sci 63: 2792.

3. Murillo-Cuesta, S. et al. (2009) Melanin precursors prevent premature age-related and noise-induced hearing loss in albino mice. Pigment Cell Melanoma Res. 23: 72.

4. Cobo, P. et al. (2009) Design of a reverberant chamber for noise exposure experiments with small animals. J App Acoustics 70: 1034.

5. Murillo-Cuesta, S. et al. (2010) Comparison of different aminoglycoside antibiotic treatments to refine ototoxicity studies in adult mice. Lab Anim. 44:124.

Brainstem responses following cochlear damage inducing tinnitus

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Cochlea damage by drugs and excessive sound exposure (noise) has been shown to lead to a partial loss of hearing function, and is often accompanied by hypercusis and phantom noise perception (tinnitus). While the functional cochlea damage can be well described by the threshold shift in the threshold audiogram (permanent threshold shift, PTS), the central neuro-sensory damage, and tinnitus related changes can not be easily specified. In a rat animal model, we examined the cochlea damage, brainstem function loss and tinnitus generation after exposure to noise of 10 kHz 120 dB SPL for 1 – 1.5 hours. Auditory threshold loss was monitored by brainstem response audiometry (BERA) and tinnitus was recorded by a behavioural conditioning approach. After the functional assessments, cochlea and brain tissues were dissected and examined for morphological changes as well as protein and gene expression of molecular markers shown to be typically changed in tinnitus. The correlation of noise induced cochlear damage, hearing thresholds and tinnitus experience could be studied. Since moderate noise exposure not affecting audiological hearing thresholds has been reported to nevertheless affect the IHC afferent innervation (Kujawa and Liberman 2009), auditory brainstem response waveform (peak amplitude growth functions and delays) has been analysed in detail. Our results show particular changes in the brainstem response waveforms closely correlated to the occurance and intensity of tinnitus, and will be discussed in the context of afferent auditory signal processing in the cochlea and in the brainstem.

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Developmental changes of the startle response induced by acoustic trauma in rats

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Acoustic trauma induced during postnatal development in rats by noise exposure results in anomalous processing of acoustical stimuli in the adult auditory system (Grécová et al, 2009, Bureš et al, 2010). In the present study, monitoring the developmental changes in the acoustic startle response (ASR) was used to assess differences in the maturation of the rat auditory system under normal conditions and after noise exposure during the critical period. The animals were exposed on the 14th postnatal day (P14) to a 125 dB SPL broad-band noise for either 8, 12 or 25 min. The ASRs to tone bursts (4 – 32 kHz, 70 – 120 dB SPL) were examined in 12 rat pups from P12 (approximate onset of hearing) to P30. In 12-day-old animals a reliable ASR was observed, as a rule, only to 110 – 120 dB SPL stimuli at frequencies below 8 kHz. Between days 12 and 14, tones of higher frequencies and lower intensities became increasingly effective in eliciting an ASR. ASRs in 13 – 14-day-old rats were characterized by similar ASR amplitudes for both high and low frequency stimuli. The maturation of the ASR pattern during the P15-P30 period was characterized in control and noise-exposed rats by a similar tendency of decreasing ASR latency, but with different changes in the ASR amplitude. In control animals the ASR amplitude evoked by low-frequency stimuli increased and the ASR amplitude to high-frequency stimuli decreased. In contrast to control animals, hyperactivity in response to high-frequency stimuli, combined with significantly larger ASR amplitudes to high-frequency (12 – 32 kHz) tones, was observed in noise-exposed animals during this period. This hyperactivity persisted in exposed animals until day P30 and was more pronounced in the animals that were exposed for a longer time. The results indicate that the startle reaction may be used as an objective indicator of the maturation of the auditory system, reflecting pathological changes associated with early acoustic trauma.

Grécová J, Bures Z, Popelár J, Šuta D, Syka J., Eur J Neurosci. 29: 1921-30, 2009
Bureš Z, Grécová J, Popelár J, Syka J., Eur J Neurosci., 325:155-64, 2010

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The subcellular distribution of presynaptic glycine receptors correlates with the occurrence of endogenous sources of their agonists

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Glycine is a major inhibitory transmitter in the mammalian brainstem. Its action is exerted through anion permeable glycine receptors (GlyRs), pentameric proteins composed of α_{1-4} and β subunits. Individual receptor subtypes show differential regional and developmental distribution in the nervous tissue. However, the factors that influence the subcellular location of GlyR subunits have not been fully elucidated yet. Previously, we have identified α1 homomeric GlyRs at giant glutamatergic nerve terminals called calyces of Held in the rat medial nucleus of the trapezoid body (MNTB). The goal of this study was to reveal the distribution of GlyRs in calyceal compartments using immunogold electron microscopy. GlyRs in MNTB slices isolated from adult rats were labelled with specific antibodies coupled to gold particles (IGP). Quantitative analysis of IGP distribution in 3D reconstructed terminals showed that the receptors were not randomly located at terminal branches. The density of IGP, 9.34 ± 0.85 per μm^2 (mean ± S.E.M.) on the average, was inversely related to the size of the calyceal compartments, suggesting an aggregation of GlyRs in larger branches. Surprisingly, the receptors were not concentrated arround the glutamate release zones, in contrast to what had been expected due to the function of presynaptic GlyRs enhancing glutamate release from the calyx of Held. On the other hand, we have observed IGP accumulation in calyceal compartments that were apposed to glycinergic nerve endings or the processes of glial cells. This strongly suggests that the distribution of presynaptic GlyRs is largely determined by the locations of endogenous sources of glycine agonists. The results support our functional data showing the activation of presynaptic GlyRs by glycine spillover.

Changes in calbindin immunoreactivity with aging in the central auditory system of the rat

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The disruption of neuronal calcium homeostasis and consequent molecular events affect neuronal viability and plasticity and are thought to be linked to a decline in neuronal performance with aging. Age-related changes in the levels of major intracellular calcium buffers are known to occur in different parts of the mammalian brain, including the central auditory pathway. In the present study, we describe the effect that aging has on the calbindin-expressing system of neurons in the higher parts of the rat central auditory pathway, in the inferior colliculus (IC), medial geniculate body (MGB) and auditory cortex (AC). In both young and old animals, calbindin-immunoreactive (CB-ir) cells were observed in all three examined structures. In the IC, the majority of CB-ir cells were found in the dorsal and external cortices and only sparse cells were present in the central nucleus of the IC. With aging, the number of CB-neurons decreased both in the dorsal and external cortices. In the MGB of young rats, CB-ir neurons were present in abundant numbers in both the dorsal and ventral subdivisions, while in old animals, the number of CB-ir cells in the ventral subdivision was significantly decreased. In the auditory cortex, CB-ir neurons were found in all cortical layers. In comparison with the IC and MGB, the age-related numerical decrease in the number of CB-ir neurons in the AC was non-significant. In addition to the numerical changes, age-related morphological alterations of the CB-ir neuronal bodies were observed in all three examined auditory structures. The described age-related changes in the calbindin-expressing system may contribute significantly to the deterioration of hearing function known as central presbycusis.

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Effect of the environment on the immature rat auditory system affected by noise during the developmental period

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During the early postnatal development of rats, the functional maturation of the auditory system strongly relies on the natural character of the incoming neural activity. Previously, we found that even a brief noise exposure of 8 min of juvenile rats on the 14th postnatal day (P14) has a serious impact on frequency selectivity and the representation of intensity in colliculus inferior neurons in adulthood (Grecova et al., Eur J Neurosci, 2009; Bures et. al., Eur J Neurosci, 2010). However, this type of noise exposure did not produce any permanent hearing threshold shift (TS). To find out whether the effect of a permanent TS results in more severe changes in the activity of colliculus inferior neurons, the noise exposure on the 14th postnatal day was extended from 8 min to either 12 min or 25 min. The present work was specifically aimed at describing the effects of prolonged noise exposure on hearing thresholds and to test the possibility of compensating for impaired hearing function during development by exposing the animals to an acoustically enriched environment (EE).

Hearing thresholds were assessed on the basis of auditory brainstem responses (ABRs), the physiological state of the hair cells were estimated by recording the distortion products otoacoustic emissions (DPOAEs). Noise exposure of 12 or 25 min resulted in an immediate increase of hearing thresholds by 10 – 50 dB at different sound frequencies. One day after the noise exposure, the rats were placed for two weeks in specific environmental conditions: they were exposed 12 h a day to complex acoustic stimulation consisting of a broad-band rippled noise of 65 dB SPL intensity with six embedded behaviorally relevant signals (with intensity 70 dB SPL), three of which triggered a glucose water release. After the end of their stay in the EE, the animals exposed to EE sounds displayed worse hearing thresholds than did control animals. We therefore decreased the intensity of the EE signals to 55 dB SPL for the rippled noise and 60 dB SPL for the embedded signals. After two weeks the rats subjected to an acoustic environment with reduced intensity showed significantly better thresholds in over the medium frequency range of 4 – 16 kHz in comparison with rats kept under standard housing conditions.

The results demonstrate that the threshold shift resulting from noise exposure during the period of early development can be partially reduced by exposure to an EE of moderate intensity. However, a higher intensity EE might result in the worsening of the hearing threshold shift.

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Comparison of the electrophysiological membrane properties of layer V pyramidal neurons in the primary auditory cortex and belt area of the rat

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Our previous results showed clear difference in the electrophysiological characteristics of neurons from different auditory cortical fields. The greatest difference was found between neurons located in the primary auditory cortex (AI) and those in the surrounding belt area. The aim of this study was to confirm our previous findings also on the intracellular level. We focused on investigatin differences in the electrophysiological membrane properties of neurons from the A1 and the belt area.

Based on extracellular in vivo recordings we distinguished the position of the AI and belt areas and denoted their borders in each rat. The whole-cell mode of the patch-clamp technique was then used to characterize the electrophysiological membrane properties of identified layer V pyramidal neurons from both the AI and belt area. 300 – 400 μm thick supravital brain cortex slices were isolated from 30 – 35-days-old Wistar rats. Layer V pyramidal neurons were chosen because they receive signals from the upper cortical layers and form the majority of projections to the subthalamic nuclei and a part of the cortical projections to the thalamus.

Four distinct cell types were recognized based on their repetitive action potential firing patterns and single action potential characteristics: regular-spiking slowly adapting type-1 cells (RS1), regular-spiking slowly adapting type-2 cells (RS2), regular-spiking fast adapting (FA) and intrinsically bursting cells (IB).

In order to reveal whether the firing properties of neurons from the auditory areas depend on the presence and magnitude of a hyperpolarization-activated inward current (Ih), we performed a series of voltage clamp, as well as current clamp experiments by perfusing slices with the well established antagonist ZD7288. Our results revealed that neurons from the AI have a significantly higher amplitude and density of the hyperpolarization-activated current than those in the belt area.

Hearing preservation in patients with Schwannoma of the 8th cranial nerve

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Schwannoma of the 8th cranial nerve is one of the most common cause of retrocochlear hypacusis. Patients with this disease suffer mostly from a triplet of symptoms: hypacusis, tinnitus and vertigo. Such a combination of symptoms suggests that the tumor influences both the supracochlear structures of the auditory pathway, as well as the labyrinth of the inner ear. Surgical removal is one of the possible treatments of vestibular Schwannoma. Improvement in microsurgical techniques should result not only in the complete removal of the tumor but also in maximal hearing preservation after the surgery.

In this study, we evaluated hearing parameters in patients with vestibular Schwannoma, who were surgically treated and examined in the Department of ENT and Head and Neck Surgery of the 1st Medical Faculty, Charles University from January 1st 2008. For audiological examination, high frequency audiometry (Madsen Orbiter 922 VL), otoacustic emissions (ILO 292 Echoport) with or without contralateral suppression and auditory brainstem responses ABR (Viking Quest) were used.

Our results show a significant decrease in the amplitudes of transiently evoked otoacoustic emissions with and without contralateral suppression in the ear with Schwannoma when compared with the healthy ear before surgery. This finding supports the hypothesis that the hypacusis in these patients is caused both by suppression of the supracochlear structures and by cochlear dysfunction. In all surgically treated patients with postoperatively preserved hearing, transiently evoked otoacustic emissions were present in the ear with Schwannoma prior to the operation. However, 50% of patients with postoperative deafness in the operated ear had TEOAE present in the ear with Schwannoma prior to the operation. Our data further support the idea, that the presence of otoacoustic emissions in the ear with Schwannoma before the surgery is a significant factor influencing the postoperative preservation of hearing in the affected ear.

Crispin van de PASSE (1564 -1637)
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